The Effect of Different Weaning Strategies on Piglet Performance and Immune Function

Thesis

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THE EFFECT OF DIFFERENT WEANING STRATEGIES ON PIGLET PERFORMANCE AND IMMUNE FUNCTION

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I declare that this thesis has been composed entirely by myself and that it has not been accepted in any previous application for a degree. The work, of which it is a record, has been done by myself. Quotations have been distinguished by quotation marks and sources of information have been specifically acknowledged.

Mary Allen

ABSTRACT

The weaning period in commercial pig production has been a continuing problem for both the piglet and producer. An investigation was carried out to assess the effects of different, relatively simple, management strategies on the stress associated with the weaning period in terms of piglet performance, behaviour and immune status. Three of the main areas identified as playing a key part in the stress associated with weaning were mixing of unfamiliar piglets, relocation to a new environment and adaptation to a new diet. Management strategies were designed relating to assess these factors.

The studies highlighted that mixing piglets pre-weaning at 14 days of age was a positive step to aid the transition of weaning, in terms of performance and immune status and requires little adaptation of current farrowing systems. Mixing at either 7 or 21 days of age does not show the same improvements in post-weaning performance.

Remaining in a familiar environment was not beneficial, and may possibly be detrimental, to the newly weaned piglet and indeed, relocation to a novel environment appears to be important for the piglet psychologically and motivates the piglet to explore its new environment and search for alternative food sources.

The benefits of creep feeding still needs further consideration in order to determine the optimal feeding strategy in relation to feed intakes pre- and post-weaning combined with the effects of creep feed on the underdeveloped digestive tract.

Although it is not possible to completely remove the growth check that occurs post-weaning, it is clear that management strategies can be utilised to reduce the stress associated with weaning and improve post-weaning growth rates.

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LIST OF ABBREVIATIONS

BSA	Bovine serum albumin
Con A	Concanavalin A
CP	Crude protein
DE	Digestible energy
DM	Dry matter
EDTA	Disodium ethylene diamine tetra-acetate dihydrate
EE	Ether extract
ELISA	Enzyme linked immunosorbant assay
IFN-γ	Interferon gamma
Ig	Immunoglobulin
KLH	Keyhole limpet haemocyanin
Mab	Monoclonal antibodies
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NDF	Neutral detergent fibre
PBS	Phosphate buffer saline
PBS/T	Phosphate buffer saline with 0.05% tween 20
PIC	Pig Improvement Company
PWM	Pokeweed mitogen
TCM	Tissue culture medium

CHAPTER 1. LITERATURE REVIEW

1.1 INTRODUCTION

Weaning is usually a gradual process and under semi-natural conditions it occurs at approximately 16-20 weeks of age in pigs (Jensen and Recén, 1989). However, in commercial situations it takes place abruptly at approximately 4 weeks of age and is usually confounded by additional challenges such as mixing with non-litter mates and relocation into a new, usually barren, environment (Puppe *et al.*, 1997). This causes several changes that affect the performance of the pigs including: the loss of the security of the sow and, often, siblings; a complete change in diet; a new environment; and increased aggression (Varley, 1995). All of these factors occurring simultaneously cause weaning to be a very stressful time for the piglet.

The impact on performance is of economic importance to the producer as it causes a reduction in weight gain or loss of weight (Okai *et al.*, 1976). This growth check comes at a time when the pig should be rapidly depositing body tissue and therefore increases the time it takes for the pig to reach it's market weight (Fraser *et al.*, 1998).

When discussing weaning the term 'stress' has been frequently used yet few authors have tried to define or quantify this ambiguous term. Stress appears to affect behaviour, performance, immune and endocrine function, all of which can be used as subjective measures/indicators of stress (Hicks *et al.*, 1998). Abnormal behaviour is often exhibited, along with impaired immune function and an increase in disease susceptibility. It is obvious that the effect of stress compromises the health and performance of each individual in different ways.

As so many changes are occurring together at weaning it is difficult to determine the relationships between these factors and the stress each factor causes. It is necessary to minimise the variability of these factors and attempt to assess the effect of each different change on the performance of the pig.

1.2 NEONATAL DEVELOPMENT

The management of the weaning period in commercial situations can often begin much earlier than the actual time of weaning. Therefore, it is important to understand the ontogeny of the neonate (newly born) and how different management earlier in the piglet's life may be able to improve the weaning process and post-weaning growth potential. The survival of the piglet pre-weaning is an essential area of understanding as this may predetermine the effect weaning has on the piglet (Varley, 1995). The development and growth of the pig is also a vital area to be understood if improvements in performance and welfare are to be made.

1.2.1 Piglet Survival

The survival of newborn piglets is highly variable (Pond and Houpt, 1978; Milligan *et al.*, 2001) and related to many factors such as genotype, birth weight, maternal investment, thermoregulation, behaviour and development, all of which important factors to consider. The majority of piglet deaths (≈50% of pre-weaning mortality) occur in the first week post-partum (English and Morrison, 1984; Dyck and Swierstra, 1987; Tuchscherer *et al.*, 2000). One of the main reasons for piglet death in the first week is overlaying of piglets by the sow. Aumaitre and Le Dividich (1984) observed that the number of overlain piglets increased with poor suckling response and malnutrition. The newborn piglet needs to be warm and clean to increase it's chances of survival during this critical neonatal period (Svendsen and Steen Svendsen, 1997).

An important consideration when identifying neonatal mortality is the incidence of stillbirths. It has been observed that many stillbirths occur during parturition (intrapartum) (English and Morrison, 1984) rather than pre-partum and therefore the time of parturition needs more attention in an attempt to reduce the number of intrapartum stillbirths. The major cause of intrapartum stillbirths is asphyxiation during delivery or in later born piglets caused by anoxia (English and Morrison, 1984). It has been determined that 70% of intrapartum stillbirths occur among the last three piglets to be born and there is a higher incidence in protracted farrowings and larger litters as well as in older sows probably due to poor muscle tone (English and Morrison, 1984; Dyck and Swierstra, 1987). Dyck and Swierstra (1987) identified eight major causes of death pre-weaning – stillbirth, crushing by sow, starvation, euthanasia, exposure, congenital abnormalities, disease and unidentified causes.

Environmental temperature plays an important part in piglet survival, as the lower critical temperature for a newborn piglet is 34°C (Mount, 1968). Therefore if the environmental temperature falls below this temperature then the piglet will be exposed to cold stress (English and Morrison, 1984). Cold stress often leads to reduced movement and smaller piglets will be more at risk as they will lose body heat at a much faster rate (Herpin and Le Dividich, 1995) leading to increased risk of overlaying by the sow, especially when piglets lie close to the sow for heat. It is therefore essential to provide a good environment with dry bedding and a heat lamp to keep the piglet safe, away from the sow in a warm environment (English and Morrison, 1984)

Tuchscherer *et al.* (2000) studied the traits of newborn piglets to identify which were more likely to survive the first few days of life. Surviving piglets were compared with piglets that died within 10 days after birth and determined that piglets which survived were born earlier in the birth order, reached the udder and took colostrum earlier and maintained a higher rectal temperature one hour after birth.

Significant relationships between risk of death and birth weight, litter size and the variation in birth weight have been found (van der Lende and de Jager, 1991). They also identified that piglets with low birth weights within the litter, especially those under 1 kg, had a very high risk of death regardless of status within the litter.

Management factors controlled by the stockperson such as the environment, attendance during farrowing and cross fostering are also important in reducing the piglet mortality in this neonatal period. The attendance of stockmen during farrowing is a luxury many farmers cannot afford especially as many sows farrow during the night (Kingston, 1989). However, Kingston (1989) proposed that there may be a cost benefit of having someone present during farrowing which is obviously easier in a batch farrowing system when all the sows are due to farrow over a short period of time. The presence of a stockperson enables attention to be given to weaker piglets by drying and aiding the piglet to suckle soon after birth (White *et al.*, 1996). This improves the piglet's chances as it allows the piglet to maintain it's body temperature and receive vital colostrum. White *et al.* (1996) found that having a stockperson in attendance at farrowing gave benefits of 1.1 extra piglets per litter and improved growth performance up to 21 days of age with piglets having been attended at farrowing weighing 5.33 kg compared to piglets unattended weighing 5.09kg at 21 days of age (*P*<0.05, Between subject mean square error 2.2).

The effects of poor farrowing conditions, pen accommodation and nursing environment have been identified as some of the most important factors that affect the survival of the piglet pre-weaning (Aumaitre and Le Dividich, 1984). Farrowing and pen conditions such as use of straw, farrowing rails, slatted floors all affect the survival rate of the piglet early in life. The use of straw appears to give the lowest percentage of losses in the farrowing house (Table 1.1) whilst slatted floors resulted in most overlaying (Barnett *et al.*, 2001). It was also determined that inadequate flooring in the farrowing pens may be the primary cause of piglet mortality from overlay and also a secondary cause when associated with other factors including susceptibility to splay-leg, entrapment of claws in slatted floors, chilling and overlaying due to starvation/weakness (Barnett *et al.*, 2001).

Table 1.1 *Comparison of the efficiency of different indoor farrowing systems for sows*

Performance	Type of farrowing system			
	Straw	Non-insulated concrete slatted floor[a]	Cages[b]	Farrowing rails[b]
No. born alive	9.5	9.2	10.9	11.0
No. at 8 weeks	8.4	6.2	8.8	8.1
Total loss (%)	11.6	32.6*	19.3	26.4*

[a]: Aumaitre, 1971 Source after: Aumaitre and Le Dividich (1984)
[b]: Robertson *et al.*, 1966
*: P<0.01 between treatments

It has been observed that piglets prefer to lie close to the sow in the period immediately after farrowing to maintain body temperature (Hrupka *et al.*, 1998) and have close access to the milk supply without having to expend too much energy on movement. The use and position of heat lamps has been studied as a method of encouraging the piglets to move away from the sow, in an attempt to reduce overlaying in the first few days post-partum. However, Hrupka *et al.* (1998) reported that during the first three days post-partum heat lamp position did not affect piglet survival or lying position of the piglet.

A review by Barnett *et al.* (2001) reported the factors involved in piglet mortality and concluded that the causes such as savaging, crushing, starvation and illness are not mutually exclusive and may be affected by other factors such as previous experience, age, nutrition, health and injury status. All of these elements affecting piglet mortality need to be taken into consideration if any improvements in piglet survival during this critical period are to be made.

1.2.2 Teat Order

The development of a stable teat order is essential for the survival of piglets and if stable teat orders were not formed weaker piglets would be unable to compete for teats and cause an increase in piglet mortality (Rosillon-Warnier and Paquay, 1984). Position of teat on the sow (anterior vs. posterior) has been reported to affect growth rates as the anterior teats have been suggested to be more productive (Dyck *et al.*, 1987). However, it has also been suggested that there is no difference in productivity of teats but that larger piglets tend to occupy the anterior teats and are better at stimulating the teat during the pre-and post-massage phases of suckling which increases milk production (Jeppesen, 1982). The process of determining the teat order has been described as having four main phases (Graves, 1984).

The first of these four phases is known as the "Teat Seeking" phase and involves the piglets searching for the warm and soft tissue of the udder (Graves, 1984). This first stage occurs almost immediately after birth and the piglets become active very quickly. There were very few or no agonistic interactions observed between the piglets during this initial phase (Graves, 1984). Piglets appear to be unable to find the teats visually (Hartstock and Graves, 1976; Graves, 1984) and rely on exploring areas with their nose. Hartstock and Graves (1976) observed that the piglets could pass a teat just millimetres away during this exploratory behaviour with their nose and still not respond to it, thus supporting the hypothesis of using smell and touch rather than sight in the early stages of development (Christison *et al.*, 1997). The later born piglets also seem to be attracted to, or by, the piglets born earlier (Welch and Baxter, 1986). This could possibly be due to vocalisation by the older piglets giving the successive pigs an indication as to where the teats are.

The second stage of this developmental process is called " teat sampling"; this stage begins once contact has been made with the udder and can last between two and six hours. The piglets begin massaging the udder with rapid head movements and then move from teat to teat (Graves, 1984). The movement from teat to teat is aided by the initial teat contact as the piglets remain at the correct height to find the next teat along

(Hartstock and Graves, 1976). Agonistic interactions during this phase are likely to occur if littermates come into contact with one another and a display of mutual aggression may be observed. The defending piglet will attempt to guard the teat by making it inaccessible to the other piglet (Hartstock and Graves, 1976). Figure 1.1 shows the number of fights observed by Hartstock and Graves (1976) during the initial phases of teat development.

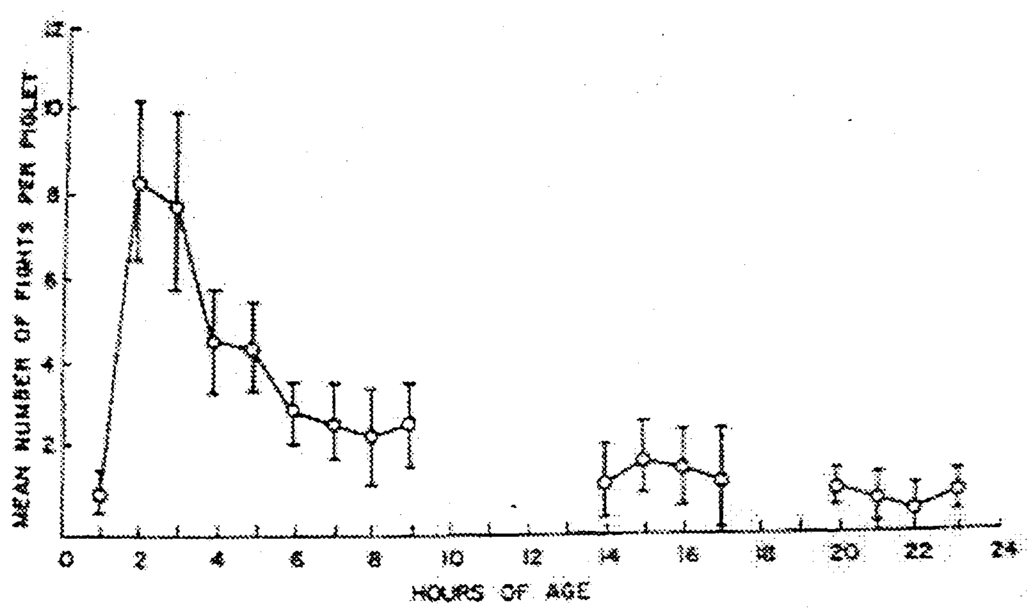

Figure 1.1 Mean and 95% confidence limits for number of fights per piglet by hours of age. Source: Hartstock and Graves (1976)

The next stage is known as "teat defence" and Graves (1984) describes this as the period of time when activity is limited to a small part of the udder and a preference for a specific teat is formed. The aggression during this stage is much less than the previous stage but any agonistic interactions are observed between adjacent littermates and any littermates that have failed to find a teat in the previous stages. If a piglet loses it's specific teat to another piglet it often refuses to suckle from any other teat and may continually fight for its teat. As the piglets grow they are less likely to suckle other teats and often starvation occurs as other teats are not functioning or are suckled by another piglet (McBride, 1963).

The final stage is " teat maintenance". This is classed as when there are no more contests between individuals for teats, teat specificity has been achieved and each piglet suckles from a specific teat resulting in the piglets fall into a routine of suckling and sleeping (Graves, 1984). The whole process can take several days for the litter to develop a stable hierarchy (Orihuela and Solano, 1985) and Figure 1.2 shows a representation of the entire process from birth to a stable teat order.

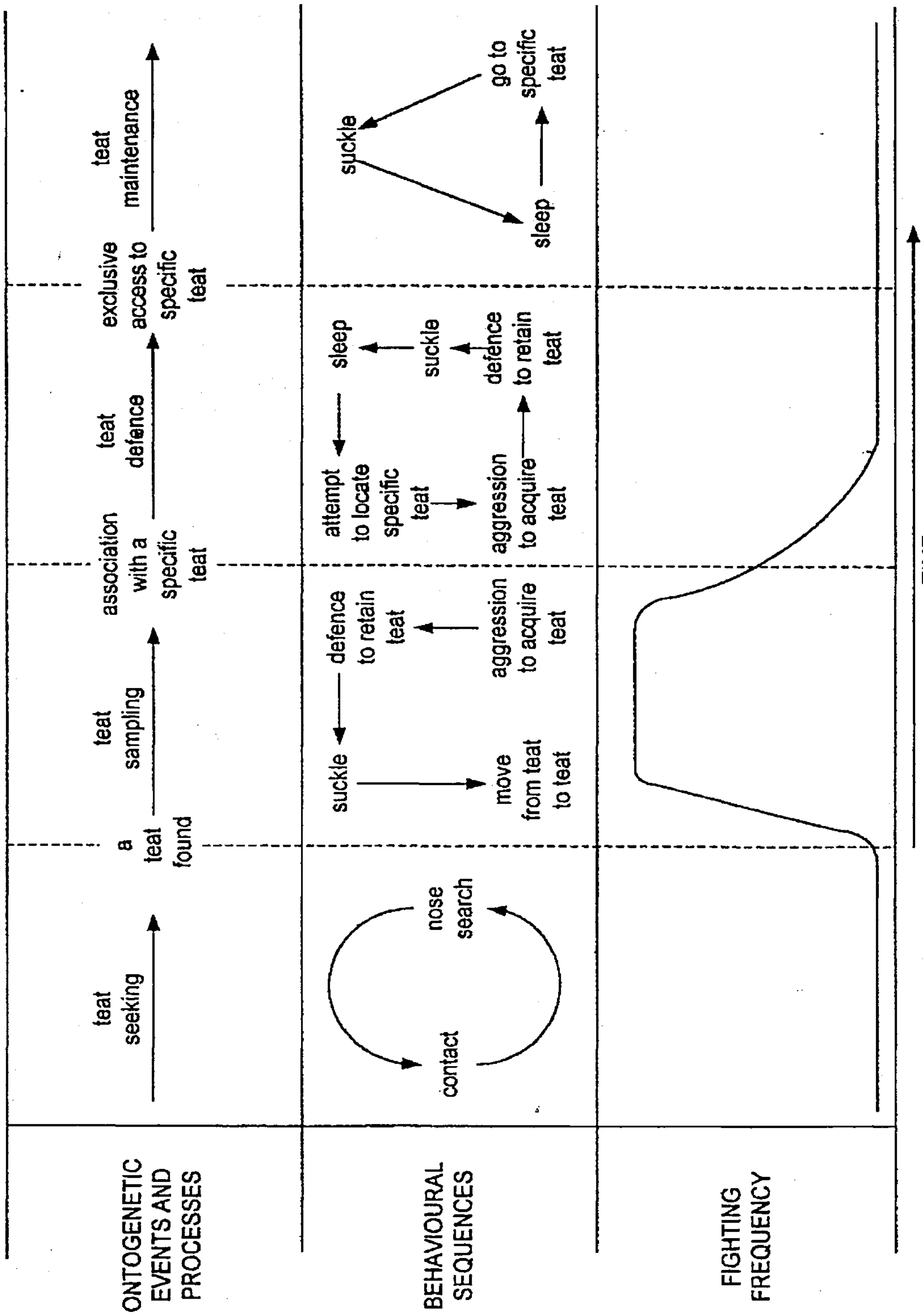

Figure 1.2 Representation of the entire teat order process

Source: Hartstock and Graves (1976)

There are a number of factors that are involved in determining the position of a piglet within the teat order. Hartstock *et al.* (1977) related birth weight and the percentage of fights won to teat position and identified several possible pathways to explain this relationship (Figure 1.3). Another factor that appears to alter the teat order is the sow and the frequency with which she turns over. This turning by the sow in the early stages of teat order development increases the time it takes for the stable teat order to be formed (McBride, 1963).

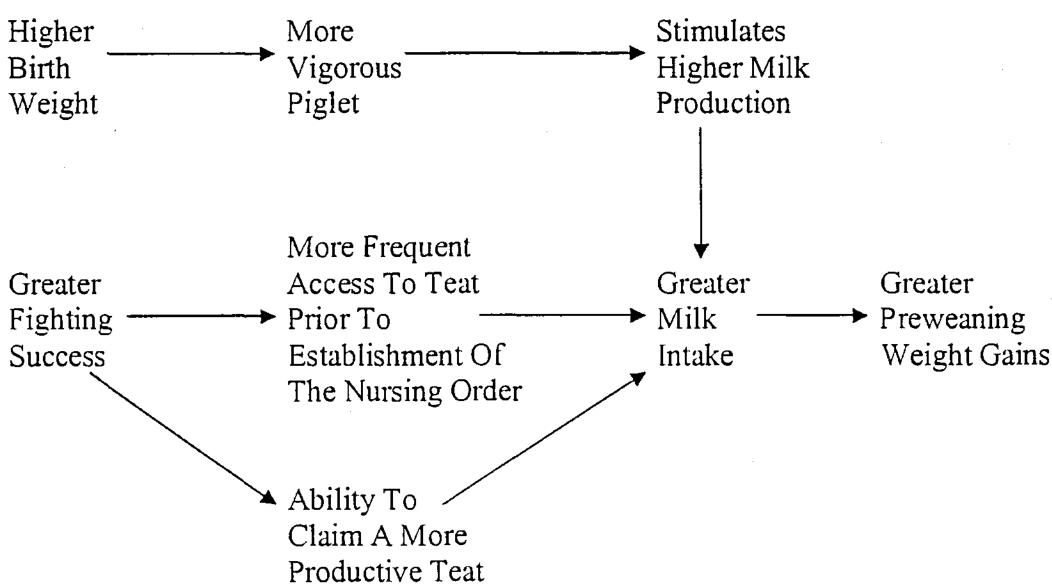

Figure 1.3 Possible pathways explaining the relationship of higher birth weight and greater fighting success to preweaning weight gain.

Source: Hartstock *et al.* (1977)

It is important to take into account the effect of size of litter and size of piglets within that litter. Litters that are growing faster (i.e. on a more productive sow) compared with a slower growing litter are seen to have a more stable teat order and lower incidences of fighting and lesion scores on the head and face (Winfield *et al.*, 1974). This has also been observed when comparing smaller litters with larger litters (Fraser, 1975).

There have been numerous reports relating to the link between teat order and social hierarchy in growing pigs (Meese and Ewbank, 1973; Scheel *et al.*, 1977). If this link is correct then it has been proposed that it may be advantageous to maintain piglets in litter groups through to slaughter to minimise the agonistic interactions that occur whilst the dominance hierarchy is being formed (Ewbank, 1976).

1.2.3 Social/dominance hierarchy

"Dominance" has many different definitions and is a keyword in many social behavioural studies (Drews, 1993). It is important to identify these definitions and the role dominance actually plays in behaviour. Drews (1993) identifies thirteen different definitions of dominance and produced an overall structural definition, which can be applied to many situations and adapted to individual studies according to the subject and theory used.

"Dominance is an attribute of the pattern of repeated, agonistic interactions between two individuals, characterised by a consistent outcome in favour of the same dyad member and a default yielding response of its opponent rather than escalation. The status of the consistent winner is dominant and that of the loser subordinate" (Drews, 1993).

It is also important to define the difference between dominance status and dominance rank. Dominance status refers to the individuals status within a dyad i.e. dominant or subordinate, where as dominance rank refers to the individuals position in a dominance hierarchy as a number or high/low within the hierarchy (Drews, 1993).

The initial period of fighting observed immediately after pigs have been mixed is believed to be due to the establishment of a dominance hierarchy (Brouns, 1993). The dominance hierarchy is the stabilising feature of a group that prevents serious agonistic encounters between individuals by the use of subtle signals (Rasmussen *et al.*, 1962; Appleby, 1983).

When unfamiliar pigs are housed together the establishment of the dominance hierarchy begins almost immediately with pairs of pigs fighting intensely. These encounters can continue for up to 30 minutes between two individuals (Meese and Ewbank, 1973) and it is common for several paired encounters to occur at any one time. This intensive fighting can continue for up to 24 hours (Ewbank, 1976) but Meese and Ewbank (1973) stated that the dominant pig is usually apparent to a human observer within 30-60 minutes of the pigs being mixed. These levels of aggression appear to reduce dramatically after the first hour post-mixing (Symoens and Van Den Braude, 1969).

Meese and Ewbank (1973) proposed that the initial dominance hierarchy formed was the one which gives precedence to rank in obtaining food and that fighting was rarely observed unless food resources are limited (Rasmussen *et al.*, 1962). The dominance hierarchy not only affects priority to food but also other resources such as space (McCort and Graves, 1982).

Once the hierarchy within a group has been established it is relatively stable with the most dominant and most subordinate pigs maintaining their position (Rasmussen *et al.*, 1962). Although it has been observed that the pigs in the middle ranks appear to change position frequently and these middle ranking pigs show regular aggressive interactions (Meese and Ewbank, 1973). The stability of the hierarchy is dependent on two factors:

1. The ability of pen mates to identify each individual in the group.
2. The continuous reinforcement of dominance by the higher ranking individuals (Rasmussen *et al.*, 1962).

The size of group may have an effect on the stability of the hierarchy as the more pigs in a group the less able the pigs will be to identify their pen mates. Stookey and Gonyou (1998) determined that as pigs are able to recognise piglets post-weaning based on their familiarity during early life rather than genetic relatedness therefore it would be beneficial to keep pigs in groups with littermates or previously acquainted piglets.

Social rank within the hierarchy is dependent on many factors such as previous experience, physical conformity, sex, environment, disease and identity of other group members (Meese and Ewbank, 1973). Beilharz and Cox (1967) stated that males were more dominant than females yet other studies have found no correlation between sex or weight with position in the hierarchy (Meese and Ewbank, 1973). These differences may have been due to the different breeds used or the different techniques used to measure dominance hierarchies within the groups. Meese and Ewbank (1973) noted a lower incidence of aggression in pigs reared outdoors therefore indicating that environment has an effect on behaviour. It is also important to identify which behaviours should be observed to determine dominance hierarchies as often different behaviours will give different dominance hierarchies (Boyd and Silk, 1983).

1.2.4 Piglet growth

The development of the newborn piglet is rapid and therefore its requirements for growth also change quickly (Svendsen and Steen Svendsen, 1997). The concept of growth is central to pig production and the development of the animal occurs not only by an increase in size but also a change in the proportions of tissues (Hammond, 1932). During the growth of an animal there is a sequence of waves of growth of different tissues (Figure 1.4) which develop at different rates (Lawrence and Fowler, 1997).

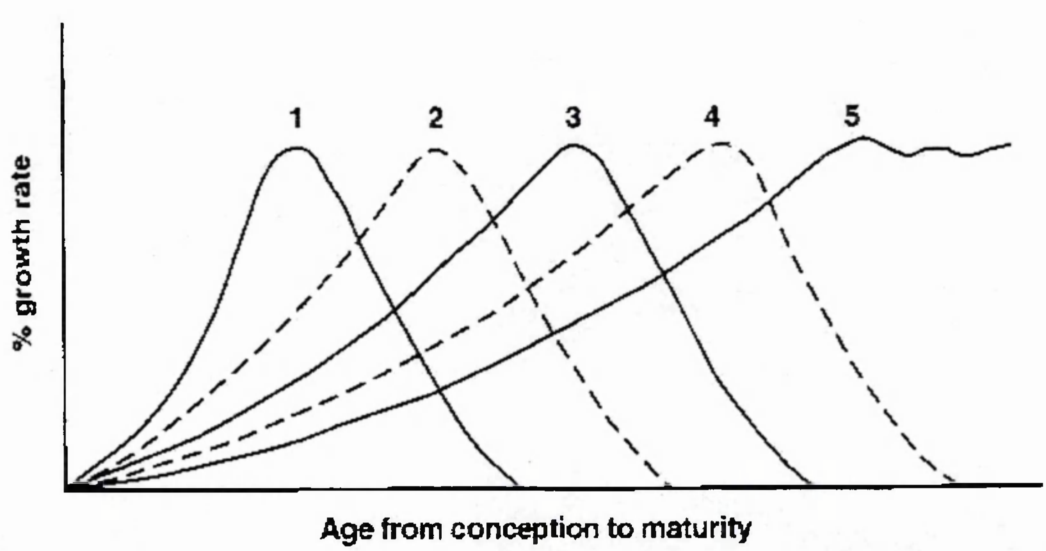

Figure 1.4 Hammond's waves of growth: 1=nervous tissue, 2=bone, 3=muscle, 4=fat, 5=daily feed intake. Source: Lawrence and Fowler (1997)

Growth is highly dependent on the feed intake of the piglet and therefore the term 'efficiency' is often used when discussing growth of pigs. Efficiency relates to the conversion of feed into body tissue and hence is directly associated with cost of production.

When considering the growth of a piglet a sigmoid growth curve can be used and it can be seen that weaning has a big impact on the potential of this growth curve due to the disturbance in growth rate usually observed post-weaning (Figure 1.5). Generally, from 30 kg to maturity the growth curve of a pig is thought of as being linear and only the gradient alters dependent on the sex of the individual (English *et al.*, 1996). Therefore, for the producer, it is more important to concentrate on the area of depressed growth caused by the weaning process and attempt to improve the performance of the piglet during this critical period.

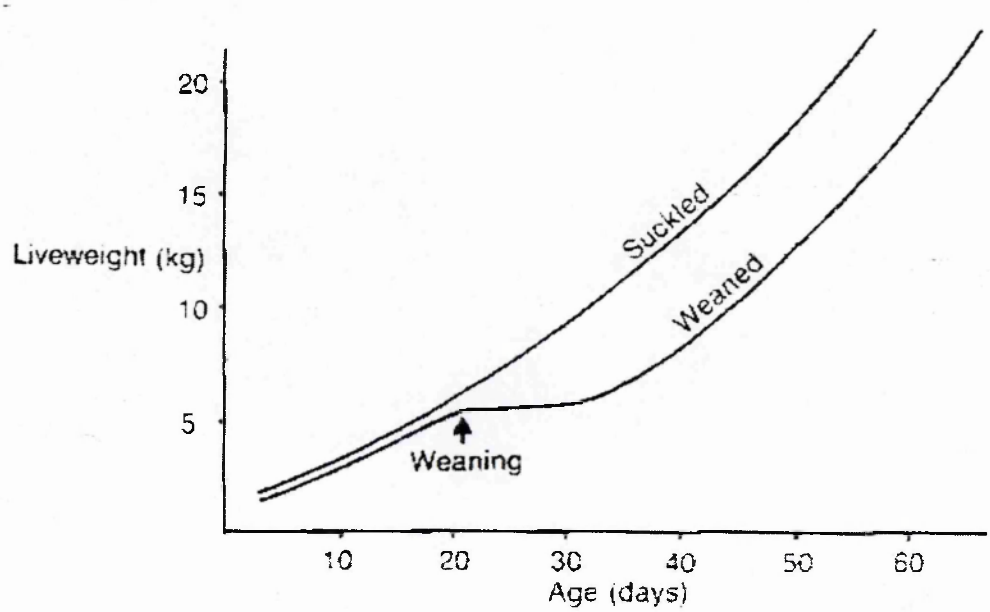

Figure 1.5 Growth curves of young pigs Source: English *et al.* (1996)

Restricted feeding can have significant effects on the growth perfomance of piglets and therefore unrestricted feeding post-weaning is recommended as reported by Tullis & Whittemore (1986) who found reduced growth rates of piglets aged 25-70 days when given restricted feed from 25 days of age until day 55 then an unrestricted diet until day 70 (Table 1.2). This indicated the detrimental effects of a restricted feeding regime post-weaning (Tullis and Whittemore, 1986). Growth is also affected by environmental temperature and stocking density as these have direct effects on voluntary food intake (Whittemore and Green, 2001).

Table 1.2 *Growth rates of weaned piglets (g/day) following restricted feed intakes and fed to appetite from 25-70 days of age.*

Days of age	Fed to appetite	Restricted 25-55 days
25-40	321	192
40-55	532	162
55-70	601	508

Source: Tullis and Whittemore (1986)

Growth of the young weaned piglets can be described by means of the Gompertz function in context to overall growth using the following equation, where the growth coefficient (B) generally ranges between 0.010-0.015 depending on sex and genotype (Whittemore and Green, 2001):

Daily gain = liveweight * B * ln(weight at maturity/liveweight)

Lawrence and Fowler (1997) discusses benefits of the Gompertz equation as it allows for both the accelerating and decelerating phases that occur during growth and it's ability to predict the weight of an animal. Growth is closely related to feeding and therefore to production costs and efficiency. It is an important area for the producer to continually try to improve performance around the weaning time. Uninterrupted growth immediately post-weaning is only observed under the highest standards of nutrition, husbandry and a good bodyweight pre-weaning (Whittemore and Green, 2001).

1.3 WEANING

The time of weaning is a critical period in the life of the pig where many changes occur causing distress and a loss in growth potential (Day and Webster, 1999). This period is of major concern to the producer, with regards to the piglet's performance, health and welfare. It is essential to gain a better understanding of this period and how management practices can be improved to aid the pig during this stressful time. The term 'weaning' has been given many different definitions over time including those looking at abrupt weaning in a commercial situation and more natural weaning in semi-natural conditions. Therefore it is important to define the meaning of weaning (Brown, 1964).

1.3.1 Definition of Weaning

Martin (1984) defined weaning as "a brief period during mammalian ontogeny when offspring switch from their mother's milk to solid food as a source of nutrition". The term weaning incorporates other aspects that are important to consider, and will be discussed further in section 1.3.2.3, such as the environmental changes and the breaking of the mother/offspring bond.

Under natural conditions, "weaning in wild species is a slow process where the mother begins to consistently reject her offspring and it can take several weeks for the offspring to adjust to the separation" (Hart, 1985). The length of weaning varies greatly between and within species.

When considering weaning in the commercial environment the term "early weaning" is often applied. This is defined slightly differently as it is not considering total independence from the mother by choice but rather by the removal of the milk supply prior to the time when weaning would normally occur (Brown, 1964).

Weaning is a period of change for both the sow and piglets that can cause significant behavioural changes, that affect the performance of the animals (Fraser *et al.*, 1998). Early removal of piglets is often seen on commercial pig farms but weaning age varies between units (Gonyou *et al.*, 1998; Worobec *et al.*, 1999) and impacts on performance. Therefore it is essential to find the optimal time for weaning to reduce any potentially detrimental effects weaning may have on the performance of the piglets.

1.3.2 The Weaning Process

The weaning process for commercial pigs is completely different to that in their natural environment and the major differences between the natural weaning process and the practice used on commercial farms are briefly illustrated in Table 1.3.

Table 1.3 *Aspects of weaning of domestic pigs under typical commercial conditions and under natural conditions*

FEATURE	TYPICAL COMMERCIAL WEANING	NATURAL WEANING
Frequency of Nursing	Remains high for several weeks	Declines more rapidly
Solid Food Intake	Relatively abrupt change	Steady increase during lactation
Separation from Mother	Sudden	Gradual reduction in contact
Introduction to New Environment and Animals	As late as 4-5 weeks, Sow absent	About 10 days, Sow present

Source: Fraser *et al.* (1998)

1.3.2.1 Natural Weaning

Natural weaning is never observed on commercial pig units as the maternal bond between mother and her piglets can last for several months (Jensen and Stangel, 1992). This is of little benefit to the producer whose main aim is to produce the maximum number of pigs each year. To maximise output of the sows it is necessary to remove the piglets from the sow as early as possible.

The natural weaning of piglets has been observed in semi-natural environments (Jensen and Recén, 1989, Jensen and Stangel, 1992, Jensen, 1995) and usually takes places between 15 and 22 weeks. It is a gradual process over several weeks during which no sudden or extreme behavioural changes were observed (Jensen and Stangel, 1992) and no distinct point was observed that could be defined as the start of weaning (Jensen and Recén, 1989).

During the period prior to complete independence from the sow, the piglets become sufficient at foraging and finding an alternative source of nutrients to their mother's milk. Jensen and Recén (1989) reported a very regular decrease in the number of sucklings over time and an increase in time spent feeding over the same period (Figure 1.6). The proportion of sucklings terminated by the sow increased from 0 to almost 100% from the first week of lactation to the fourth week and remained at this high level throughout the rest of the lactation period.

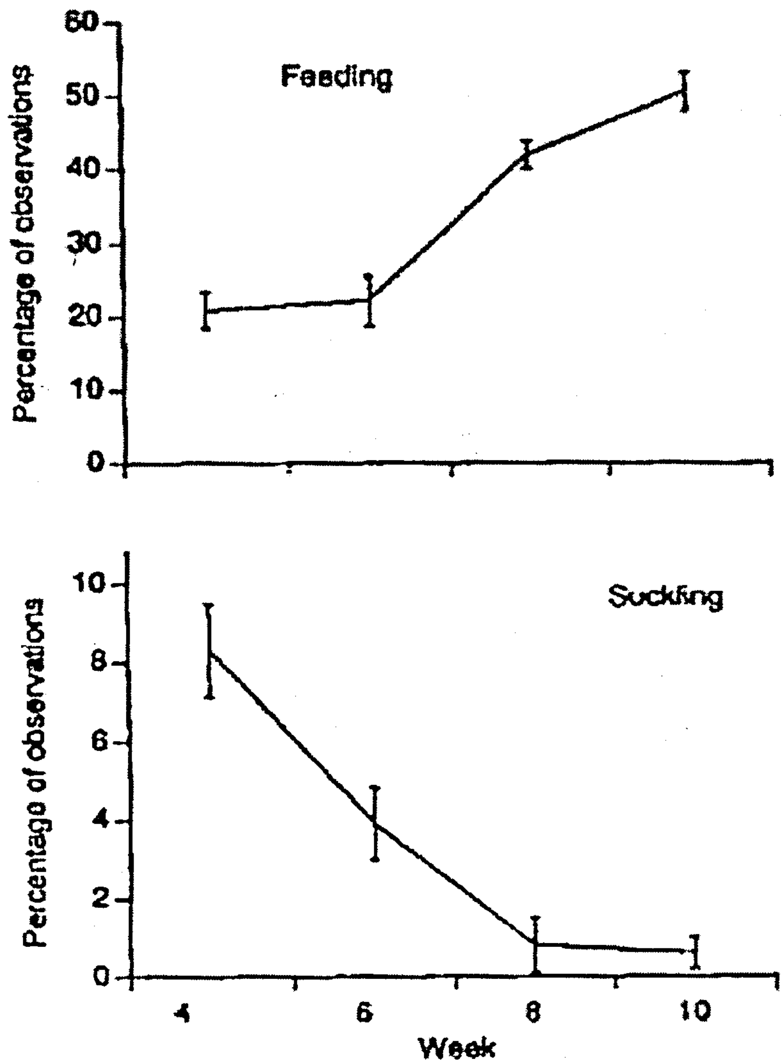

Figure 1.6 Average percentage of observations in each observation week (± SE) in which focal piglets were feeding and suckling (Note differing scales). Source: Jensen (1995)

Weaning of individuals within a litter takes place at different times (Jensen and Recén, 1989) and this appears to be due to the piglets suckling the less productive teats receiving less benefit from suckling and therefore finding alternative sources of nutrients earlier than the piglets on the more productive teats.

1.3.2.2 Commercial Weaning

In commercial practice the weaning process has become a single step rather than a number of steps over a period of time. The term 'weaning' is used to define the abrupt separation of mother and offspring which takes place before the time when natural weaning would occur between the sow and her piglets (Brown, 1964).

English *et al.* (1996) reviewed work carried out with the aim of finding the optimum age for weaning piglets without compromising the reproductive potential of the sow. The following major factors needed to be considered when determining the optimum age for weaning were trends in sow milk yield, immunity gap that occurs around 2 to 3 weeks of age, weaning to oestrus interval and conception rate, subsequent litter size, housing costs, food costs, attitude of labour and level of husbandry skill available and whether the pig unit is new or established.

All of these factors have to be carefully considered and the optimum weaning age is dependent on finding a balance between the advantages and disadvantages between each factor. In the UK there has been a distinct preference to wean piglets at three or four weeks of age, however, current research (DEFRA Project code: ISO212, Sustainable systems for pig weaner management: AGEWEAN, 2003-2007) is beginning to assess the effect of weaning at 6-8 weeks of age in an attempt to improve the performance of piglets post-weaning. It is clear that many of the factors are related to the sow and the speed with which the sow can be re-served again without reducing subsequent litter size and minimising costs (English *et al.*, 1996).

1.3.2.3 Factors involved in the weaning process

The weaning process can be split down into five main factors that are thought to be the main stressors, which result in decreased performance in the immediate post-weaning period. It is generally thought that these factors in combination cause major strains on the individual piglet and therefore compromise their growth (Varley, 1995).

The five main stressors are: -

a) Breaking maternal bonds

b) Relocation to a new environment

c) Social stress

d) Nutritional change

e) Disease susceptibility

It may be possible to minimise the stress of weaning by improving one or several of these factors or by separating the factors so that there is a time delay between them. Algers *et al.* (1990) split weaning into two different periods: - firstly, the removal of the sow at six weeks post-partum (classed as weaning); and secondly, mixing the piglets (known as regrouping) seven days later. It was evident from the results that both factors affected the performance of the piglets and that the frequencies of aggression towards other piglets and to some extent submission during agonistic interactions all increased. The feeding and drinking behaviours were also affected by both periods with increased incidence of each behaviour being observed. This gives an indication that removing the sow alone at 6 weeks of age still shows a similar change in behaviour indicative of stress to that caused by a change to the environment.

This approach of separating out the management factors has also been reported to be a useful method of quantifying to what extent each factor plays in causing the detrimental changes in growth and behaviour and how improved management practices may minimise stress and maintain growth rates (Varley, 1995).

a) Breaking of maternal bonds

The reaction of the piglets to the loss of their mother involves both physiological changes and behavioural patterns that are indicative of distress (Fraser *et al.*, 1998). The most obvious effect of the removal of the sow is the deprivation of the piglets with a familiar presence. It has also been observed that the piglets miss the oral and nasal gratification provided by suckling (Fraser *et al.*, 1998). The frustration from this loss of suckling behaviour appears to induce behavioural problems such as tail biting and belly nosing, which are detrimental to the health and welfare of the piglets exhibiting these behaviours and those receiving them (Dybkjaer, 1992).

Algers *et al.* (1990) found that removing the piglets mother at six weeks post-partum induces similar behavioural changes to those seen when piglets are regrouped a week later. This indicates that loss of the mother causes similar responses as those seen at weaning due to relocation and regrouping.

The removal of the sow means a complete change of lifestyle for the piglet. During the natural process of weaning the sow slowly introduces these changes in the piglet as it develops into an independent individual, but in commercial situations the sow has no opportunity to aid the development of the piglets in this way (Bøe, 1991). One of the major changes is the loss of milk and this causes gastric changes that would not normally occur at this stage in development. It is apparent that the effect of maternal separation on the piglet is an important emotional element that needs further research to help understand the physiology of it completely (Fraser *et al.*, 1998).

b) Relocation to a new environment

The post-weaning environment has a large influence on the expression of abnormal behaviours in the newly weaned piglet (Bøe, 1993). It has been reported that when newly weaned piglets are moved to barren environments there was an increased frequency and intensity of unnatural behaviours (Bøe, 1993). It was also associated with increased aggression, which may lead to skin lesions thus allowing the potential entry for pathogens through the skin barrier (Hessing *et al.*; 1994).

Pigs are commonly moved to new environments several times during their life and, at each move, unfamiliar pigs are grouped resulting in the development of a new social hierarchy (Hessing and Tielen, 1994; Varley, 1995). It was thought that environmental enrichment of each new environment, e.g. using novel items, decreases the aggression between individuals due to novel objects giving the piglets an alternative object to explore and release their frustration on and enables relocation to be a positive management tool not a stressor (Schaefer *et al.*, 1990). The introduction of playthings such as tyres or chains has been advocated and is used more frequently on a commercial basis to minimise the stress of relocation. However, it appears to be important that familiarity with the inanimate object reduces the effectiveness of the object to enrich the environment (Schaefer *et al.,* 1990). It is difficult to separate out the factors of social stress and change of environment as these two factors, in commercial situations, often occur together.

Bøe (1993) looked at the effect of weaning at two different ages (4 and 6 weeks old) into two different environments and concluded that some behavioural changes were observed in the younger animals but that the time of weaning had little effect if the piglets were moved to an enriched environment. This indicated that the piglet's attentions were drawn away from one another to the bedding or toys used to enrich the environment.

Housing systems used post-weaning can vary greatly in floor type, the use of straw and hiding places; all having effects on the behaviour and performance of the pigs. McKinnon *et al.* (1989) demonstrated that health and welfare was improved when pigs were on solid flooring and with small quantities of straw available compared to fully perforated or part-solid floors. Bøe (1993) identified that in flat decks which are considered barren environments, explorative behaviour was still expressed but redirected towards other penmates rather than the environment. This has been reported to be indicative of compromises to the animal's welfare, which may lead to a decline in the performance (Bøe, 1993) and Puppe *et al.* (1997) determined that the newly weaned pig appears to have more trouble adapting to a new environment than coping with unfamiliar pigs.

c) Social stress

The mixing of non-littermates is usually standard practice in most commercial situations yet it is still one of the main stressors associated with weaning, leading to fights between unfamiliar individuals to determine the dominance hierarchy of the newly formed group (Petherick and Blackshaw, 1987; Francis *et al.*, 1996; Otten *et al.*, 1997). In a commercial situation, mixing is generally carried out to split sexes and equalise body weights to give uniformity to the pen (Blecha *et al.*, 1985; Gonyou, 1997). This allows the smaller pigs to be managed differently on more appropriate diets without having to compete with larger individuals for the resources.

Blackshaw *et al.* (1987) looked at the effects of different group compositions on the performance and behaviour of piglets and identified that mixing caused very high levels of agonistic behaviour. This agonistic behaviour was due to the pigs having to fight to form a new dominance hierarchy. The study showed that the group comprising of four piglets from three litters gave the least number of agonistic interactions when compared to combinations of piglets from two or three litters, however, no apparent differences in production data was reported. However, Spicer and Aherne (1987) reported effects of group size and stocking density on performance of newly weaned piglets.

Pluske and Williams (1996a) stated that the most probable cause of psychological stress was when non-littermate piglets fight after being placed in new social groups. Aggression after weaning is one of the major factors associated with depressed growth rates, causing the growth check and therefore mixing of unacquainted piglets should be kept to a minimum (Petherick and Blackshaw, 1987).

Pluske and Williams (1996b) reported that co-mingling piglets at approximately 10kg prior to weaning partially overcomes the stress related to weaning. Some unpublished data (cited by Fraser *et al.,* 1998) claimed that early mixing of piglets at 10 days of age pre-weaning resulted in better weight gains after the piglets were weaning at four weeks of age compared to pigs from intact litters that were not mixed prior to weaning.

In group housing systems, where sows and their litters are together in one large pen, it has been observed that sows have problems identifying their own litter which often results in the disruption of suckling and the poor milk letdown (Pedersen *et al.*, 1998). It also appears that the piglets are unable to identify their mothers when grouped together. Wattanakul (1997) studied this system to try and determine how grouping piglets at 11 days of age affected suckling behaviour, agonistic behaviour and performance. This was also done in conjunction with sow relocation 10 days later to a different farrowing crate. It was concluded that grouping had no adverse effects on the long-term growth of the piglets but by three weeks after weaning, there was a trend for piglets that had been grouped prior to weaning to be heavier compared to those that had not. Relocation of the sow caused a high incidence of cross-suckling and increased agonistic behaviour of piglets both pre- and post-weaning (Wattanakul, 1997).

Social facilitation has been described as a behavioural phenomenon where the presence of piglets feeding encourages other piglets to feed as well (Keeling & Hurnik, 1996). Mixing unfamiliar piglets can cause the loss of social facilitation that occurs between piglets that are familiar with each other and cause a decrease in food consumption, so if pigs are kept in familiar groups, it may be possible to increase feed intake by social facilitation. In their studies on social facilitation, Keeling and Hurnik (1996) reported that although social facilitation was observed in female pigs, it was not the case in male pigs. This has implications on trough space and feeding regimes when considering mixed sex groups of piglets post-weaning.

Stookey and Gonyou (1998) reported that familiarity between piglets affected aggression levels and that agonistic interactions are usually observed more frequently between unfamiliar individuals. This indicates that mixing before weaning may increase the number of individuals that are familiar with each other and may decrease aggressive encounters through recognition of familiar individuals.

d) Nutritional challenge

The nutritional change associated with weaning has profound effects on piglet performance (Varley, 1995). The change from a milk diet to a solid feed at commercial weaning is sudden and at a time when the piglet's gastrointestinal tract is immature and unable to cope. As well as this change in diet the combined stress of weaning often causes the piglets to stop eating completely for several days (Bruininx *et al.*, 2001) which also has an adverse effect on the gastrointestinal morphology by altering the villous height and crypt depth thus reducing absorptive capacity.

Although piglets often begin to eat solid food from approximately three weeks of age, intake by individuals is incredibly variable (Fraser *et al.*, 1998). Many experiments have been undertaken to try to improve the piglets' adaptation to a post-weaning diet by feeding diet prior to weaning (e.g. Okai *et al.*, 1976; Fraser *et al.*, 1994) but these have been met with varied success. Pajor *et al.* (1991) concluded that individual variation of solid feed intake in the suckling pig was related to birth weight, where high birth weight pigs ate more creep feed when suckling compared to low birth weight pigs.

Currently in the UK, 'The Welfare of Farmed Animals (England) (Amendment) Regulations 2003' state that piglets should not be removed from their mothers before 28 days of age unless the welfare or health of the dam or her piglets would otherwise be adversely affected. However, piglets can be weaned up to 7 days earlier if they are moved into specialised housing which is thoroughly cleaned and disinfected between each group of piglets. Therefore, commercial weaning generally occurs between 24 and 28 days. The age of the piglet at weaning has a measurable effect on the amount of food that can be eaten and the efficiency with which it is utilised. Crenshaw *et al.* (1986) looked at the effect of age and concluded that three week old pigs can eat and utilise more food than two week old pigs (405g/day vs. 338g/day for the complex diet, SE 20, $P<0.01$). This is likely to be due to the gastrointestinal tract having longer to develop and therefore as weaning age increases so does the ability for piglets to adapt to new diets.

The subject of piglet nutrition and weaning is vast and is covered in more detail in section 1.6.

e) Disease susceptibility

Piglets are born immunologically naive; they have no circulating antibodies and also have a limited ability for lymphocyte responses (Kelley, 1980). They rely on maternal antibodies being transferred via colostrum during the first 12 hours of life. This passive immunity provides protection from pathogens for the neonatal period allowing time for the adaptive immune system to develop. However, the weaning process currently occurs at a difficult time in the ontogeny of the piglet's immune system; maternal antibody levels are decreasing and their innate and adaptive immune systems have not been fully developed (Griffin, 1989).

One key concern of commercial producers is to what degree does the stress on piglets at weaning make individuals more susceptible to disease. Kelley (1980) and Griffin (1989) reviewed the effects of stress on immune function and the main point that was identified was that adverse environmental situations (e.g. weaning, mixing) affect the animals resistance to disease by altering the activities the cells of the immune system. Blecha and Kelley (1981) observed decreases in antibody production and also in the effectiveness of the cellular immunity (Blecha *et al.*, 1983). Both of these changes will cause a depression in resistance to disease. This alteration to the immune function may compromise the effects of vaccinations that are initiated at weaning as the synthesis of specific protective antibodies could be limited (Kelley, 1982).

From a review by Gaskins (1998) it can be seen that immunology in the pig has been assessed in limited studies, which suggests that disease challenge during stressful periods, such as weaning, causes high economic losses. The effect of weaning on immunity is discussed in further detail in section 1.5.

1.3.3 Alternative weaning strategies

There are several alternative management strategies that have been researched, such as the family system, multisuckling environment and the freedom crate. All these attempt to improve piglet survival and viability and improve the piglets chances post-weaning by giving them a better start to life whilst also improving sow welfare (McGlone and Blecha, 1987; Hatet *et al.*, 1994).

The first alternative system is the family system where sows remain in a group throughout gestation and lactation and the piglets remain with the sows until approximately 12 weeks of age (Hatet *et al.*, 1994; Goetz and Troxler, 1995). This system has several advantages including higher growth rates of piglets (Figure 1.7), lack of a growth check (Hatet *et al.*, 1994), maintenance of the sows social group with freedom for movement (Goetz and Troxler, 1995) and longer, more frequent suckling bouts (Arey and Sancha, 1996).

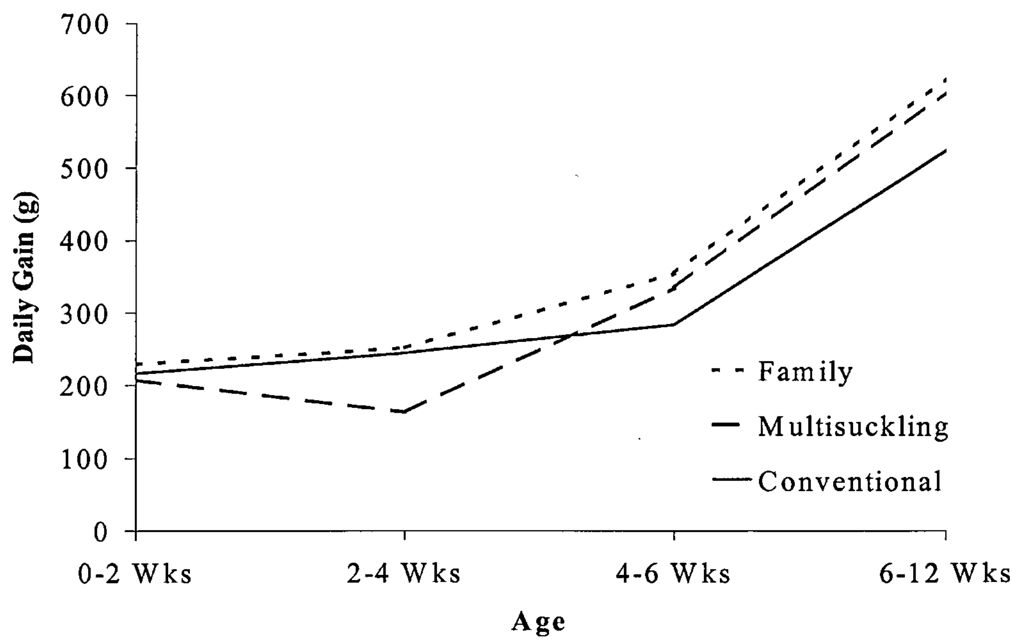

Figure 1.7 The effect of various indoor weaning methods on growth rate.
Source after: Hatet *et al.* (1994)

These advantages show that this system is capable of improving the performance of the piglets and potentially improving the welfare of the sows. However, there are a number of disadvantages which have been observed such as a high incidence of cross-suckling (Goetz and Troxler, 1995). It is more difficult to maintain a constant high temperature in an open family system compared with the conventional farrowing crate. The open family system limited the movement of piglets around the pen because they remained under the heat lamps (Arey and Sancha, 1996). The other area for concern considered by Goetz and Troxler (1995) was the aggression by the sows during nest-building. Overall this system works best outdoors because of the increased amount of space required to give the sows sufficient space to maintain their litters and also reduce the risk of aggression over nest space.

Another system used is the multisuckling system, which is similar to the family system in that the sows are group housed although this is only during lactation (Wattanakul *et al.*, 1997a; Weary *et al.*, 1999). Sows usually farrow in traditional crates and are then moved to a communal pen when the piglets are approximately 12 days old (Wattanakul *et al.*, 1997a) allowing integration of piglets at a similar age to that found in a natural environment (Jensen, 1988). The disruption of grouping post-farrowing on suckling behaviour has been attributed to increased aggression at the udder caused by cross-suckling (Edwards, 1993; Wattanakul *et al.*, 1997b). Piglets from this system have been reported to adapt to weaning better by fighting less and have a smaller growth check in the first week post-weaning compared with piglets conventionally reared (Edwards, 1993; Pajor *et al.*, 1999).

Grouping often leads to aggression between the sows while they establish a social hierarchy (Bryant and Rowlinson, 1984) within the group. Growth checks are observed in both the conventional system and the multisuckling system although they occur at different times (after weaning and grouping respectively) (Wattanakul *et al.*, 1997a).

Another alternative system often used is the Freedom crate such as the Liberty crate manufactured by Quality Equipment (Figure 1.8). This allows the sow freedom but maintains the individual pens of sows and litters (Arey, 1993). The design allows farrowing to occur within a crate but then, after several days, opening the crate allows the sow to move around within a pen area whilst giving the piglets a creep area away from the sow for protection (Arey, 1993).

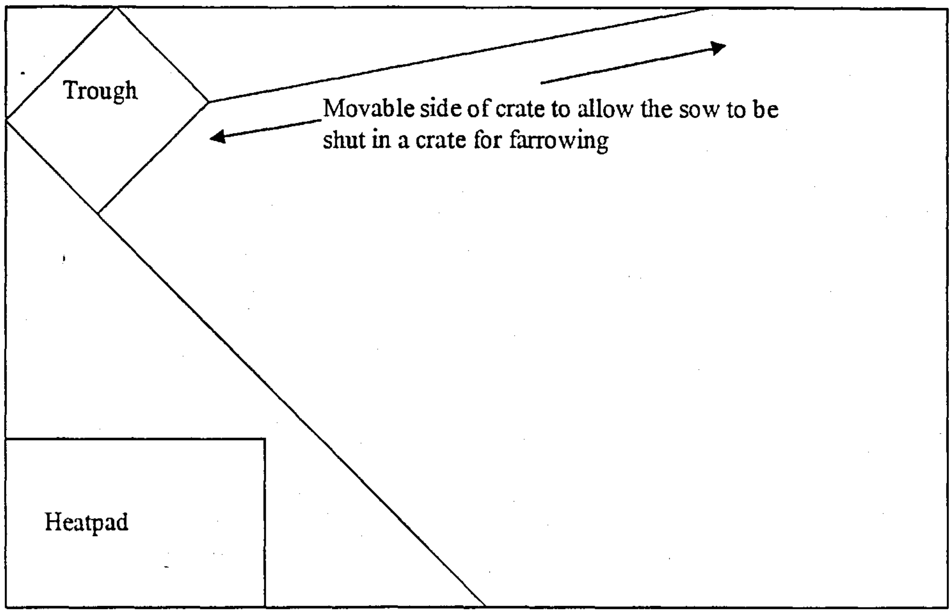

Figure 1.8 A diagrammatic representation of a freedom crate design (Quality Equipment – Liberty Crate)

These pens have been reported to reduce the incidence of piglet mortality caused by overlaying in the first few days post-partum and to improve the conditions for the sow during lactation by allowing her the freedom to move around and access to bedding (Arey, 1993).

Segregated early weaning or medicated early weaning, as it was originally called, is a more recent advancement, commonly used in the USA, in weaning management techniques (Harris, 1990). This involves the separation of piglets from the sow from 7 to 14 days *post-partum* and relocation of the piglets to an isolated site kept clean and free from pathogens (Harris, 1990). The main issue with this system is that the piglets require either a very high standard of hygiene or increased use of antibiotics to minimise mortality along with highly palatable diets (Tokach *et al.*, 1994; Nessmith *et al.*, 1996).

This system is not used widely in Europe because when weaning occurs at 7 days of age, there. are problems of increased weaning to oestrus interval and increased embryonic losses during the following pregnancy leading to reduced litter sizes (Dagorn *et al.*, 1995).

A further alternative is the specific-stress-free (SSF) housing system which allows piglets to remain in the same pen from birth to slaughter and therefore minimises the stress and improves performance parameters on the piglet by not mixing or transporting piglets between different stages (Ekkel *et al.*, 1995).

All of these alternative systems have given improvements in production and potentially improved welfare of the sow and her piglets under scientific conditions. However, once they have been applied to a commercial situation they have produced poor results and often unacceptably high piglet mortality (Edwards and Fraser, 1996).

The major factor that influences the use of these alternative systems by the producer is cost as they all involve large changes to buildings. It is, therefore, important to try to improve the management strategies of the conventional system currently being used as well as exploring the alternatives.

1.4 STRESS

The term 'stress' has many different definitions and is used to describe many different situations (Stott, 1981; Dantzer and Mormede, 1983; Dybkjaer, 1992). Within animal husbandry stress takes on many different meanings, therefore it is important to identify all of these different meanings and look at the effect of stress on animals kept in intensive conditions.

Generally, the term stress can be defined as an indication that an environmental condition is unfavourable to the welfare and health of an animal (Stott, 1981). However a more detailed definition is given by Fraser *et al.* (1975) "An animal is said to be in a state of stress if it is required to make abnormal or extreme adjustments in its physiology or behaviour in order to cope with adverse aspects of its environment and management".

Khansari *et al.* (1990) used the definition "Stress (physical, chemical or psychosocial) represents the reaction of the body to stimuli that disturb its normal physiological equilibrium or homeostasis, often with detrimental effects", whereas Dantzer and Mormède (1983) used Selye's (1956) concept to define stress as "exposure to nocuous environmental factors (stressors) elicits a nonspecific reaction. This reaction, known as stress, is characterised by enhanced pituitary-adrenal reactivity and facilitates return to homeostasis".

A more detailed explanation can be found by Breazile (1987) who defined stress as "an internal (physiologic or psychogenic) or environmental stimulus that initiates an adaptive change or a stress response in an animal." Further to this definition Breazile (1987) split the term "stress" into three different forms:

a) Eustress is good stress that concerns non-harmful stimuli allowing an animal to beneficially respond and maintain homeostasis.
b) Neutral stress causes responses that are not harmful or beneficial to the animal's well-being.
c) Distress may or may not be harmful in itself to an animal but the response that it induces is harmful to the animal's well-being and comfort. Prolonged or intense eustress or neutral stress stimuli can cause distress responses.

These definitions lead to the use of the term stress causing significant confusion as to it's meaning and therefore the definition of the term stress in this thesis is as follows: stress, whether it is favourable or not, is a stimulus which causes an adaptive response within the animal. This adaptation could be physiological or behavioural.

The number of physiological measures that have been used to measure stress includes endocrine, autonomic and changes in temperature and brain activity. Behavioural measures have been paradoxical (Levine, 1985), including both agitated and immobilised animals, vocalising and non vocalising animals considered to be stressed. It is clear to see that many of these parameters are contradictory and therefore definitions of stress need to be determined when undertaking studies involving measurement of stress.

1.4.1 Responses to stress

An animal's response to stress often initiates various disorders such as changes in feeding behaviour, hypertension, poor reproductive success or feed conversion (Breazile, 1987). These responses are generally initiated in the central nervous system involving many different pathways.

Stress stimulates several adaptive hormonal responses (Axelrod and Reisine, 1984). In the most general terms, a large increase in adrenal steroids, such as cortisol, is observed for a short time after an acute stress which is then subsequently followed by depressed levels (Stott, 1981). However, in situations where the stress is long term and often severe, the levels of adrenal steroids become subnormal and this in turn can affect the reproductive steroids (Stott, 1981). The measurement of plasma cortisol is used to reflect the body's response to different stressors (Jensen-Waern and Nyberg, 1993). However, when trying to measure stress in livestock it is difficult to assess the adrenal activity without disturbing the levels further (Baldi *et al.*, 1989; Rushen, 1991). In order to measure stress, the use of catheters and surgically modified animals has been tried to reduce the effects of continuous invasive procedures (Smith *et al.*, 2003) and researchers have looked towards physiological changes associated with this activity such as growth, immunity and reproduction.

There are two main axes important in assessing an animal's ability to cope with acute stressors, the sympathetic-adrenal medullary (SAM) system and the hypothalamic-pituitary-adrenal (HPA) cortex system (Dantzer and Mormède, 1983). There has been a suggestion that a general response to a stressor exists known as a General Adaptation Syndrome (GAS) which occurs in three main stages (Selye, 1946). The first phase is an initial alarm reaction, followed by a stage of resistance to the stressor and then biological exhaustion, potentially followed, eventually by death (Selye, 1946).

An alternative concept looks at the response from a stressor causing an increase in the activity of the sympathetic nervous system causing a release of the catecholamines, adrenaline and noradrenaline and an increase in heart rate generally reported as an acute stressor. This alternative physiological change that may occur is known as the emergency reaction or 'flight or fight' response as described by Cannon (1935) and involves the release of catecholamines to enable the individual to mobilise resources very quickly for flight or fight. If the stressor persists the hypothalamic-pituitary axis causes the release of corticosteroids and the stress becomes known as a chronic stress (Stephens, 1980).

Increased levels of ACTH and glucocorticoid hormones due to stress causes changes within the body such as delayed healing of wounds, muscle wasting, enhanced susceptibility to infection and immune deficiencies and metabolic alterations (Breazile, 1987). Other hormones such as vasopressin, oxytocin and brain opiod peptides also react to stress by interacting with brain mechanisms and in chronic stress situations lead to anorexia and disruption of reproduction along with increased cardiovascular responses, such as increased heart rate, vasoconstriction in non vital organs (e.g. gastrointestinal tract) caused by increased circulating levels of epinephrine and norepinephrine (Breazile, 1987).

Many of the stressors observed in livestock are related to the management of the environment (Otten *et al.*, 2001) and whether the understanding of how the animal adapts to environmental changes leads to improvements in production (Stott, 1981). There are distinct differences in the response to different adverse stimuli, for example glucocorticoid levels have been reported to increase with fear or anxiety, whereas pain, particularly prolonged pain, tends to result in a reduction in glucocorticoid levels (Lay *et al.*, 1992). Generally, stress responses can be placed into two categories, behavioural and hormonal, depending on how the body adapts to any environmental changes (Dantzer and Mormède, 1983; Figure 1.9).

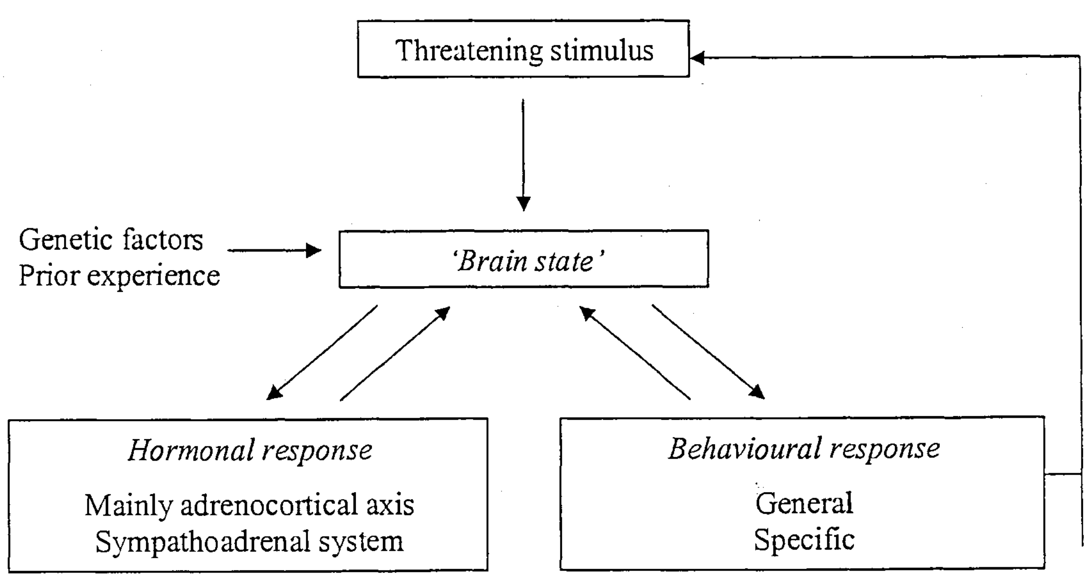

Figure 1.9 A model of the relationship between environmental threatening stimuli and the body's response Source: Dantzer and Mormède (1983)

In terms of weaning 'stress' is associated with the physiological and behavioural responses often observed post-weaning and it is in this context that the term 'stress' will be considered relating to this critical period for the piglet.

1.5 EFFECT OF WEANING ON IMMUNE FUNCTION

The newborn piglet is born with a naïve immune system due to the lack of transfer of serum antibodies from the sow through the placenta. Therefore it is essential for the piglet to gain antibodies through the sow's colostrum as soon as possible after birth to protect the piglet until it's own immune system develops (Dee, 1999).

The immunity of piglets at the time of weaning has been an area under scrutiny for numerous years as a result of the immunity gap that occurs at approximately 2-3 weeks of age which is just prior to the point of weaning for many producers in the UK. The immunity gap is the period where the maternal antibodies are declining in the sow's milk and the piglet's immune system is still underdeveloped and not capable of coping with disease; therefore the piglet is at greater risk from infection (Dee, 1999).

The relationships between stress and the immune system has been reported on by numerous authors (Kelley, 1980; Griffin, 1989) and the main theme that comes through is that many stressors can alter the susceptibility of animals to disease and cause alterations to immune status (Kelley, 1980). The immune system does not work alone; it has intricate relationships with the endocrine system and the central nervous system (Figure 1.10) when a stressor is encountered (Kelley, 1988).

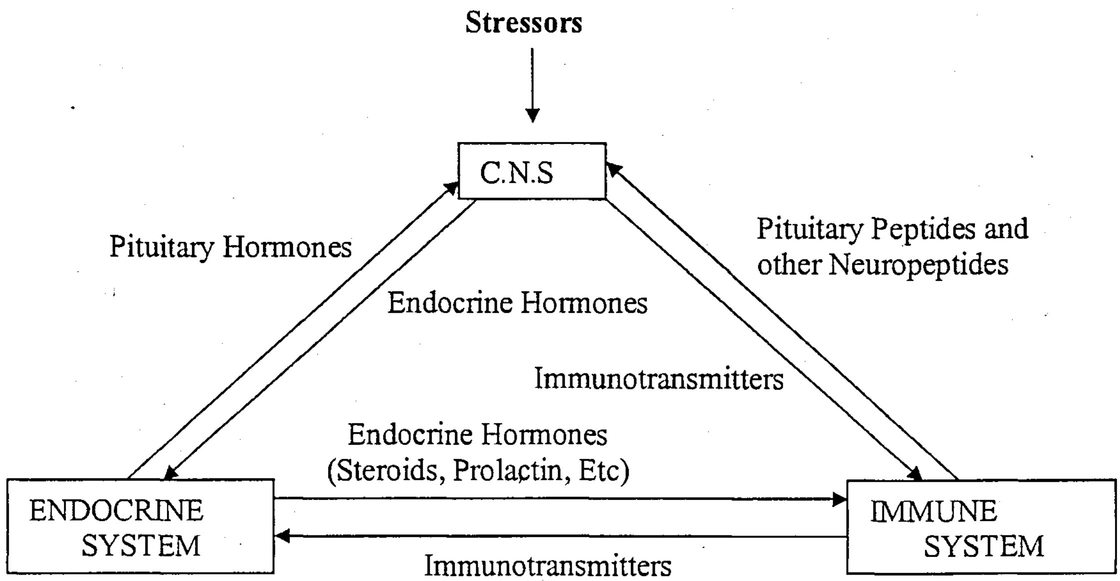

Figure 1.10 The relationships between the central nervous system (CNS), endocrine system and the immune system and the influence of stress

Source: Khansari *et al.* (1990)

One of the problems that weaning causes in terms of affecting the immune system is that it occurs prior to the full development of the immune system therefore reducing the capability to cope with the introduction to new pathogens (Blecha *et al.*, 1983). This also occurs with a combination of stressors present at weaning which has been reported to affect the immune system (Blecha *et al.*, 1983; Blecha *et al.*, 1985; Bailey *et al.*, 1992; Wattrang *et al.*, 1998).

Blecha *et al.* (1983) identified that weaning at an early age (younger than 5 weeks of age) causes a decrease in the piglet's ability to mount a cell-mediated response using the intradermal reaction to Phytohemagglutinin (PHA) and lymphocyte blastogenic response which were suppressed in piglets aged 2, 3 and 5 weeks of age. However, it was also determined that mixing piglets at weaning did not have any significant changes to the cell-mediated or humoral immune response post-weaning (Blecha *et al.*, 1985). This indicates that mixing of unfamiliar pigs, and hence additional stress at weaning, does not cause the suppression of the cell-mediated response observed at weaning.

Hessing *et al.* (1995) classified piglets based on their consistent behavioural responses into an active or a passive response and then assessed these two groups of piglets in terms of humoral and cell-mediated immune responses to stress. Piglets that were active showed a high cell-mediated immune response but a low humoral immunity, whereas passive piglets showed the complete opposite and this converse relationship is believed to be due to the switch in expression of the different subset of T-helper cells during cell-mediated responses (Hessing *et al.*, 1995). It is therefore important to remember that piglets are individuals and cope with weaning differently. Schrama *et al.* (1997) also supports the theory that individual's behaviour coping strategy strongly affects the humoral immune response with passive coping styles leading to enhanced antibody responses to a combination of novel antigens (keyhole limpet haemocyanin, ovalbumin and tetanus toxoid).

When thinking about the coping ability of individuals then size of piglet may have an effect. Smaller piglets within litters (runts) have been assessed to determine if they have a reduced ability to cope with weaning (Switala *et al.*, 1998). The results showed that these runt piglets had the same capability to cope with weaning in terms of their immune system.

It is very difficult to produce the ideal conditions for each individual within a group of piglets so the best way to determine the ideal conditions of piglets is to look at the majority and try and accommodate those piglets. The combination of weaning and changes in temperature have been identified as potential stressors. Both have an effect on the synthesis, whether it is an increase or decrease, of antibodies post-weaning (Blecha and Kelley, 1981). Cold stress is not the only temperature change to affect immunity, heat stress of temperatures up to 33°C also suppresses antibody response (Morrow-Tesch *et al.*, 1994).

The immune system also has a close relationship to gut health in it's ability to mount immune responses to both harmful and, occasionally, harmless antigens (Bailey *et al.*, 2001). The mechanisms for this immunity are not fully understood but are related to the organisation of the mucosal lymphoid tissues within the gastrointestinal tract which are not fully developed until 5-7 weeks of age and are therefore not fully effective at the time of weaning (Vega-Lopez *et al.*, 1995; Bailey *et al.*, 2001). The relationship between dietary antigens and the immune system is discussed in further detail in section 1.6.

Overall, it is clear that there are many effects of weaning on the immune system. The key point is to determine the extent to which the various stressors involved in weaning affect the piglet's immune system during this time of development.

1.6 NUTRITION AND WEANING

The nutrition of the pig is vital to it's survival throughout the various stages of production but none more so than at weaning when the most severe change of diet occurs from a liquid (sow's milk) to a solid diet (Thacker, 1999). To aid this transition many studies have looked at creep feeding to improve the piglet's digestive tract and experience of solid feeds (Aherne *et al.*, 1982; Barnett *et al.*1989; Pajor *et al.*, 1991; Appleby *et al.*, 1992).

1.6.1 Creep feed

Many researchers have tackled the area of creep feeding in piglets and the advantages and disadvantages have been discussed at length. Advantages such as better adaptation to weaning as indicated by increased feed intakes, improved weight gain post-weaning (Pajor *et al.*, 1991; Appleby *et al.*, 1991; Appleby *et al.*, 1992; Delumeau and Meunier-Salaün, 1995) and heavier weaning weights from 4 weeks onwards (Fraser *et al.*, 1994). However, a disadvantage to creep feeding is that there is the possible risk of provoking a type I hypersensitivity to dietary antigens such as soya (Miller *et al.*, 1984) or specific proteins within soya such as glycinin and beta-conglycinin (Stokes *et al.*, 1981) and this will be discussed in section 1.6.2. Kelly (1990) assessed the effect of creep feeding on the digestive tract of piglets post-weaning and highlighted that post-weaning changes in structure of the small intestine are not related to the amount of creep feed provided.

Bøe and Jensen (1995) reported individual differences between piglets and found a high correlation between weight and feed intake with larger piglets consuming more food and occupying the most productive teats. Waran and Broom (1992) reported no relationship between creep feeding and weight gain post-weaning. This supported work by Barnett *et al.* (1989) and a reduction in weaning weight of piglets consuming more creep feed was observed by Kavanagh *et al.* (1996). These results illustrate the lack of consistency in creep feeding research.

Whilst it remains uncertain if creep feed is beneficial to piglets post-weaning, an additional factor that has been reported to influence the effect of creep is the amount of creep feed consumed. Creep feed intakes vary notably both between and within litters ranging from 0-2382g/litter and 0-674g/piglet pre-weaning (Delumeau and Meunier-Salaun, 1995). Pajor *et al.* (1991) showed that total creep consumption varied from 13-1911g/piglet from 2 weeks of age to weaning and suggested that higher creep feed intakes were typical of larger, more mature piglets rather than as compensation for poor milk intake.

Wet/gruel feeding has been used to try to improve feed intake pre- and post weaning (Blanchard *et al.*, 2000; Brooks *et al.*, 2001; Corrigan *et al.*, 2002). Generally liquid feeding appears to maintain the continuity of nutrients in the period post-weaning (Deprez *et al.*, 1987) as it is more appealing and familiar where as dry feed is not consumed due to unfamiliarity to the piglets and such a drastic change of form when compared to milk.

1.6.2 Transient/Type I hypersensitivity

Transient hypersensitivity to new dietary antigens has been reported to increase crypt cell production rate which results in malabsorption and villous atrophy (Stokes *et al.*, 1986). Li *et al.* (1990) reported that soybean meal, from conventionally processed commercial diets, decreased villous height and increased serum IgG titers to soybean proteins along with decreased performance of piglets weaned at 21 days of age. This indicates that antigens may be present after processing in soybean meal used in early-weaned piglet diets. Similar results have been observed by numerous authors (Dunsford *et al.*, 1989; Li *et al.*, 1991; Miller *et al.*, 1991; Hankins *et al.*, 1992) indicating that transient hypersensitivity may play a part in the depression of growth observed post-weaning in some piglets.

It has been suggested that there is a high correlation between previous experience of dietary antigens of the mother and immune tolerance of the same dietary antigens in the offspring (Telemo *et al.*, 1991). It may therefore be important to provide these antigens to the sow to reduce the risk of transient hypersensitivity in the piglets (Telemo *et al.*, 1991).

Early weaning has a big impact on this hypersensitivity and it has been observed that oral tolerance can be developed in piglets if weaning is delayed and piglets are allowed to gradually wean themselves on to a soya based diet over a 12 week period (Miller *et al.*, 1994). Wilson *et al.* (1989) reported an initial immune response and absorbed antigens to soya proteins when weaned at 10 weeks of age but by six months the piglets no longer absorbed soya antigens when reintroduced to the diet. This supports the theory of early weaning directly affecting the tolerance of the piglet to dietary antigens (Stokes *et al.*, 1987).

The reaction to soybean meal was initially related to antinutritional factors such as lectins or protease inhibitors, however, even after heat treating the soybean meal, which should inactivate any antinutritional factors, a depression in growth was still being observed (Li *et al.*, 1990). This lead to further research relating to this problem and the identification of an increased immune reaction to various dietary antigens (Stokes *et al.*, 1981). An increase in the density of B and T lymphocytes to soya within the small intestine of both calves (Lallès and Dréau, 1996; Dréau and Lallès, 1999) and piglets (Dréau *et al.*, 1994; Dréau *et al.*, 1995) was observed post-weaning.

1.6.3 Post-weaning nutrition

After weaning, growth is limited by several constraints reviewed by Thacker (1999) which include inadequate levels of digestive enzymes, reduced absorptive capacity due to changes in structure of the small intestine, removal of sow's milk and immunological protection, dietary antigenicity, low feed/water intakes and infection. It is apparent that all these factors will affect the piglet and knowledge of how weaning affects these areas is essential in aiding the piglet during the period post-weaning (Thacker, 1999).

Makkink (1993) identified that piglets coped with weaning in one of two ways as shown in Figure 1.11, therefore it is necessary to provide the best quality diet to encourage piglets to begin eating as soon as possible post-weaning. Bark *et al.* (1986) showed that weaning piglets prior to full development of the digestive tract caused them to consume insufficient feed for maintenance during the initial three days post-weaning therefore causing weight loss and a growth check.

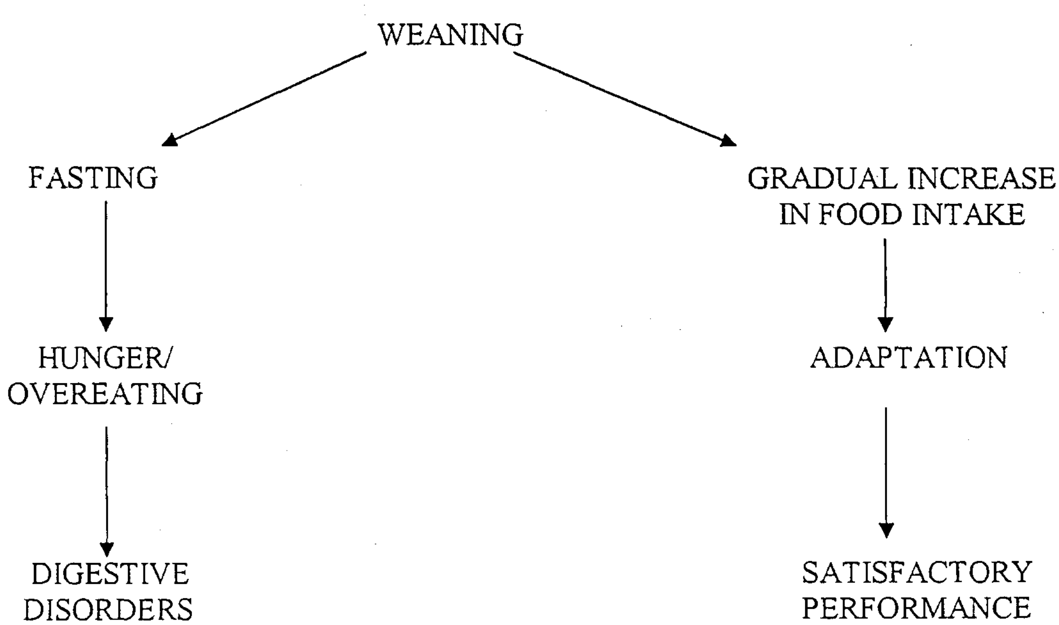

Figure 1.11 The two pathways of a piglet's ability to cope with weaning
Source: Makkink (1993)

Tokach *et al.* (1994) examined the developments in weaner pig nutrition and identified that the introduction of high nutrient density feeds and stages of starter diets improved the performance of piglets post-weaning. Toplis and Tibble (1992) reported improvements on growth rates (291 vs. 181g/day) when comparing a high digestible diet and standard starter diet for the newly weaned piglet. High digestible and palatable foods are important (Toplis and Tibble, 1992) and using several stages of diet and changing them gradually allows the transition from a milk-based diet to a cereal/soya diet. This allows the digestive tract time to adapt (Fowler, 1985).

The newly weaned piglet needs a highly palatable and digestible diet, which meets the changing nutrient requirements. Although there is a large range of nutrients required by piglets and deficiencies in any of these will have profound effects, generally there is little difficulty in providing these nutrients and they are relatively inexpensive. However, there are two key factors that affect the form and cost of piglet diets that need to be considered - amino acids and energy.

Amino acids are important in the diets of piglets as they commonly limit the performance. Lysine tends to be the most limiting amino acid in pig feeds across the world (Thacker, 1999) and as lysine concentration is increased growth rates and feed efficiencies are also improved (Table 1.4). The ratio of other amino acids to lysine is important to achieve optimal performance and the NRC (1998) recommends the following pattern of amino acids for the newly weaned piglet (Table 1.5).

Table 1.4 Lysine requirements (% total lysine in the diet) of segregated early-weaned piglets (0-14 days post-weaning)

% Lysine	1.20	1.35	1.50	1.65	1.80	1.95
Daily Gain (g)	331	362	382	408	394	402
Gain/feed	0.87	0.90	0.93	1.03	1.03	1.03

Source: Owen *et al.* (1995)

Table 1.5 Percentage of amino acids required by the newly weaned piglet (NRC).

Amino Acid	Percentage required
Lysine	100
Isoleucine	55
Methionine	29
Threonine	65
Tryptophan	18

Source: NRC (1998)

However, there is a continuing debate about the ideal levels of amino acids and research carried out in the UK (Yen *et al.*, 1986; Wang and Fuller, 1989) indicate much higher levels for certain amino acids relative to lysine, particularly methionine and cystine (Table 1.6) and it was reported that the nitrogen was utilised significantly better compared to the NRC amino acid pattern. The need to provide an ideal balance of amino acids brings about the need for multiple protein sources such as skim milk and soybean meal (van Heughten *et al.*, 1994; Thacker, 1999).

Table 1.6 Percentage of amino acids required by the newly weaned piglet in the UK.

Amino Acid	Percentage required
Lysine	100
Isoleucine	60
Methionine + Cystine	63
Threonine	72
Tryptophan	18
Valine	75
Leucine	110
Phenylalanine + Tyrosine	120

Source: Wang and Fuller (1989)

The other major factor that is of importance is energy, which is required for changes in body composition and growth (Le Dividich and Sève, 2001) and the protein to energy ratio (Lysine:DE ratio) which are often affected by the period of underfeeding observed around the point of weaning (Van Lunen and Cole, 1996). This supports the need for a highly digestible diet post-weaning to improve feed intakes (van Heughten *et al.*, 1996). The provision of energy is the most expensive part of any pig diet and the provision of this energy comes mainly from the carbohydrates, proteins and fats provided in the feed (English *et al.*, 1996).

The carbohydrates used within piglet diets can be divided into two fractions, starch that is readily digested by the piglet and dietary fibre (cellulose) which cannot easily be broken down and utilised. Starch is the major energy provider within any piglet diet (Wiseman *et al.*, 2001; Shen and Liechty, 2003) and within the processing of diets many variables (temperature, moisture and time) affect the specific structure of amylose and amylopectin, the two main glucose polymers of starch. However, Wiseman *et al.* (2001) identified that starch digestion is a relatively simple process and even at the point of weaning piglets should have the correct enzymes to break starch down for energy utilisation. Therefore it is more important that correct processing occurs not to aid digestibility of cereal starches but to improve palatability for the newly weaned piglet.

The nutrition of the newly weaned piglet is continuously under scrutiny but it is not just the diet that needs to be considered. The internal changes within the digestive tract of the piglet at weaning also affect the future performance of the piglet (Kenworthy, 1976). In order to identify these changes it is necessary to understand the structure of the digestive tract specifically the small intestine where it is known that the absorptive capacity is reduced post-weaning (Hampson, 1986a). There has been a significant change in the villi observed post-weaning (Figure 1.12). The reduced ability of the piglet to absorb the nutrients required is caused by the classic reduction in villous height observed due to the stress of weaning and dietary change (Kenworthy, 1976).

It has been observed that the villi within the small intestine undergo villous atrophy and crypt hyperplasia post-weaning (Pluske *et al.*, 1991) and the growth check and poor food intakes along with post-weaning diarrhoea that are characteristic post-weaning are related to the effect of weaning on the development of the digestive tract (Cera *et al.*, 1988; Funderburke and Seerley, 1990; Nabuurs *et al.*, 1993).

Cera *et al.* (1988) identified that villi shape changed from finger-like villi to more smooth compacted tongue-shaped villi after weaning (Figure 1.12) and that this has serious consequences on the piglet's ability to digest and absorb nutrients provided. This change in shape and reduction in height appear to help explain the piglet's increased susceptibility to post-weaning diarrhoea (Kenworthy, 1976; Hampson, 1986a).

The development of the digestive tract appears to have some interaction with nutrient intake during the post-weaning period and therefore changes may occur if there is a disruption in nutrient supply as commonly seen post-weaning (Kelly *et al.*, 1991; Fan, 2003). However, it is both the change in structure and the low levels of specific digestive enzymes that are associated with post-weaning digestive problems rather than a reduction in absorptive capacity (Miller *et al.*, 1986). These changes can be manipulated by the diet provided post-weaning as highlighted by Hampson (1986b) where the provision of sow milk replacer reduced the change in crypt depth commonly observed post-weaning and hydrolysed casein based diets reduced changes in crypt depth and enzyme brush border activity.

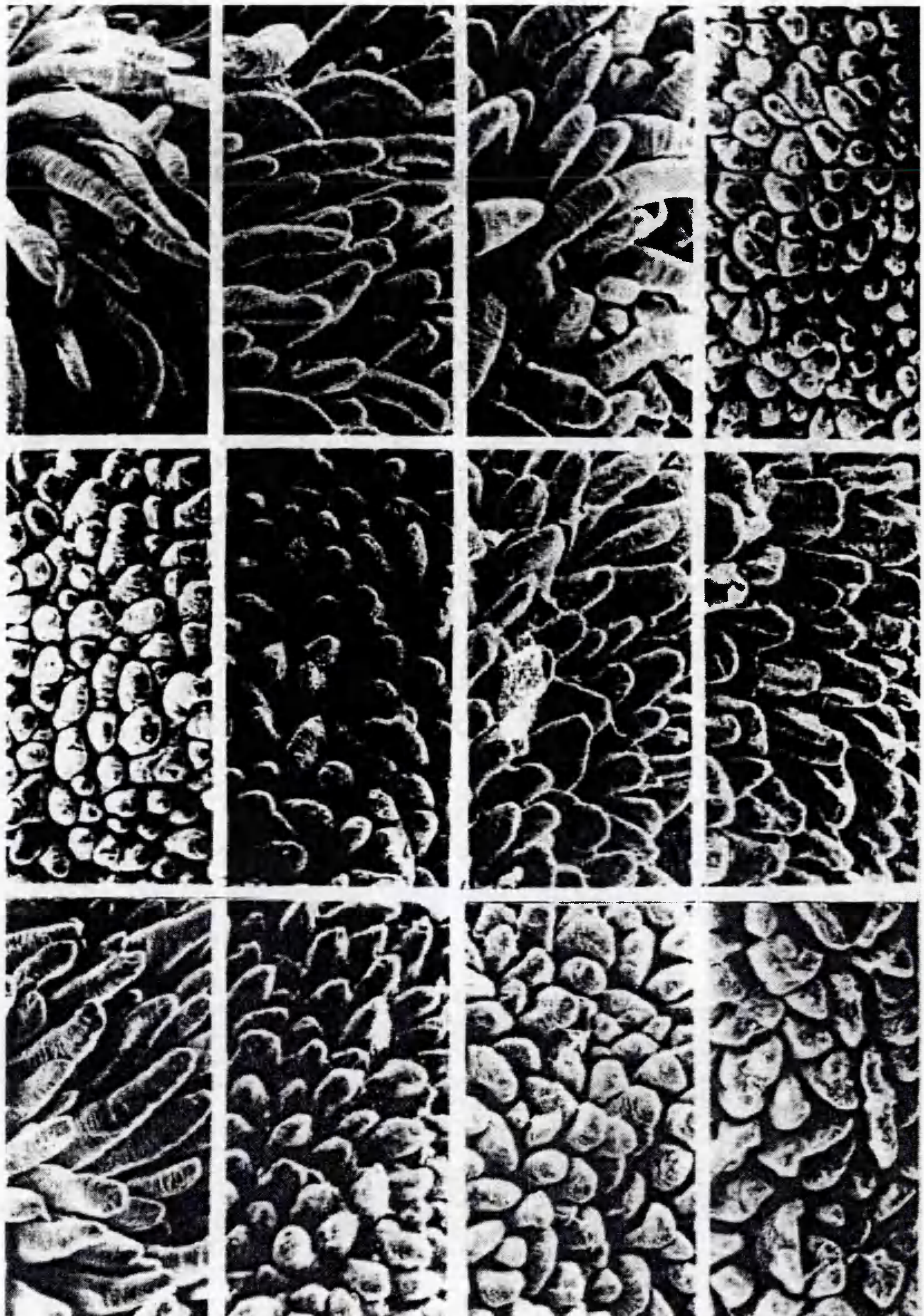

Figure 1.12 Scanning electron micrographs of small intestine villi length and morphology. (From top left to bottom right – 2, 10, 21 days of age suckling, 24 day of age (weaned at 21 days), 28, 35, 42 and 49 days of age (weaned at 21 days), 28, 35 days of age suckling, 38, 42 days of age (weaned at 35 days)). Source: Cera *et al.* (1988)

As previously discussed the limited amount of creep consumed prior to weaning may have caused a transient hypersensitivity to certain food antigens such as soya in the diet (Miller *et al.*, 1984) which may have added to the effect of weaning on gut morphology. However, Nabuurs *et al.* (1993) found that giving creep feed prevented the shortening of villi and therefore reduced the severity of post-weaning diarrhoea yet the amount of creep feed consumed plays an important part on this response post-weaning.

1.6.4 Feeding strategies

The nutrient requirements have been well researched and it is known that they change rapidly for the early-weaned piglet. Therefore, the optimum feeding strategy is to feed a number of diets over the post-weaning period to support these changes (Fowler, 1985; Tokach *et al.*, 1994). However, the piglet is constantly struggling to cope with the change of diet (liquid to solid) during this stressful period and this has lead to numerous studies outlined below attempting to improve post-weaning feed intakes by altering management techniques.

Several attempts have been made to improve post-weaning feed intakes, although little improvements on growth were observed, including weaning in the evening compared to the morning which has been reported to enhance performance (Ogunbameru *et al.*, 1992) or the use of different feeder types (Pluske and Williams, 1996c). Numerous additives such as enzymes, probiotics and flavour enhancers have been assessed to try to improve pre- and post-weaning performance with varying success and are reviewed by Li *et al.* (2003). Officer (1995) reported that multi-enzyme supplements had no beneficial and even detrimental effects on piglet performance and suggested that enzyme activity is not limited post-weaning as has been previously suggested to be the cause of decreased nutrient digestibility (Hampson and Kidder, 1986). Flavour enhancers can be utilised to encourage food intake especially if similar in flavour to the sow's milk (Li *et al.*, 2003).

Bruneau and Chavez (1995) determined that piglets were capable of distinguishing and showing preference for diets with different cereal contents with a clear preference for a wheat based diet compared to a corn, barley or oats based diet. This also supports the work by Dalby *et al.* (1995) where choice feeding was assessed as a management technique for allowing piglets to select the most appropriate diet for each piglet's individual needs. By offering a choice of diets to a group of piglets housed together these authors proposed that it may allow the piglets to differentiate and feed for their individual requirements thus removing the need to always provide for the smallest piglet within a pen. It may also remove the need to mix piglets at weaning into pens of similar sizes for the convenience of feeding the correct diet. Dams *et al.* (1994) also reported an improvement in performance and feed efficiency when piglets were offered a choice of two diets over 5 weeks post-weaning which differed in nutrient composition but not feed ingredients.

The potential for altering management strategies for the post-weaning period and incorporating some of the knowledge about nutrient requirements may help to improve the feed intake of piglets post-weaning and reduce the growth check often observed.

1.7 BEHAVIOUR AND WEANING

As it is generally difficult to assess stress and welfare, in terms of physiological response. Another method that is relatively inexpensive and easy to apply in commercial situations is assessment of behaviour (Dybkjaer, 1992). Behaviour is known to be a good indicator of stress (Fraser and Broom, 1997) and behavioural changes can be a good indication of how an animal is adapting to the changing environment. Before being able to assess an animal's behaviour as a indicator of welfare it is necessary to have a detailed understanding of a species' natural behaviour patterns and this can be regarded of as the animal in it's natural environment (Fraser and Broom, 1997). In a commercial situation, piglets have to cope with various stressors around the time of weaning and behavioural changes can be used to assess the effect of these stressors on welfare of the piglet (Fraser *et al.*, 1998).

Stereotypic behaviours such as belly-nosing and tail-biting are often observed post-weaning and this has been connected to the frustration of a piglet's motivation to suckle as discussed in section 1.2.3.2. When piglets are first weaned and relocated to flat deck accommodation they spent the initial five minutes exploring the new environment and also attempting to escape (Friend *et al.*, 1983). This exploration soon turns to chewing or other oral behaviour directed to pen mates which have been identified as indicators of stress (Dybkjaer, 1992).

Environment appears to have serious implications on the development of normal behaviour patterns (Beattie *et al.*, 1995). The effects of environmental enrichment throughout pig production has been reported (Beattie *et al.*, 1995; De Jong *et al.*, 1998; Beattie *et al.*, 2000) and it is clear that enrichment of a barren environment even with only the addition of bedding improves welfare.

Various studies (Hessing *et al.*, 1993; van Erp-van der Kooij *et al.*, 2000) have determined that piglets exhibit individual behavioural characteristics, identified by the backtest, that enable piglets to cope with stressful situations. Although Jensen *et al.* (1995) found no existence of a consistent individual behaviour characteristic in piglets using social challenge tests such as open-field tests; piglets may have different capabilities in coping that these can be assessed using behaviour characteristics. This may give an insight into how individual piglets cope with the weaning process and why certain piglets have more severe growth checks (Hessing *et al.*, 1993). Piglets which react passively to stress may facilitate easy adaptation to weaning and reduce the growth check associated with the weaning period (Giroux *et al.*, 2000).

The increase in fighting that has been observed post-weaning is a continuing area of concern with obvious implications on welfare and production (Petherick and Blackshaw, 1987). The main reason for the increased aggression observed is the mixing of unfamiliar piglets at weaning causing a need to establishment a dominance hierarchy within the group (Meese and Ewbank, 1973). Various factors have been assessed in an attempt to reduce aggression such as different temperatures (McGlone *et al.*, 1987), group size (Turner *et al.*, 2001), as discussed in section 1.3.2.3 and sedatives (Friend *et al.*, 1983). However, there has been little success in using heat stress as a method for reducing agonistic encounters and temperatures above 32°C reduces submissive behaviours which may have negative effects on aggressive behaviour (McGlone *et al.*, 1987). Sedatives, such as amperozide and azaperone, have proven to be more successful in the early stages of mixing piglets but only delays the onset of aggressive encounters and often causes vomiting (Tan and Shackleton, 1990; Barnett *et al.*, 1993).

Generally, it has been observed that weaning can cause changes in a piglet's behaviour. Therefore it is an important area to consider when assessing different management strategies around the weaning period and the use of behavioural, performance and physiological parameters should be used to assess the overall effects of stress caused by weaning.

Many of the studies reported are related to piglets at a variety of ages but due to the nature of this project it is important to highlight the key studies relating to weaner piglets between 3-4 weeks of age (Table 1.7).

Table 1.7 *List of studies relating to piglets weaned between 3-4 weeks*

Author	Subject
Algers *et al.* (1990)	Regrouping and behaviour at weaning
Bailey *et al.* (1992)	Immune response following early weaning
Bark *et al.* (1986)	Meal intervals and weaning on feed intake
Barnett *et al.* (1989)	Creep feed and weaning
Blackshaw *et al.* (1987)	Group composition and weaning
Blecha & Kelley (1981)	Cold and weaning stress on immune status
Blecha *et al.* (1983)	Immune status and weaning
Bøe (1993)	Weaning age and environment
Bruininx *et al.* (2001)	Feed intakes and group housing of weaner piglets
Bruneau and Chavez (1995)	Dietary preferences
Cera *et al.* (1988)	Weaning and digestive physiology
Christison (1996)	Aggression at weaning
Close and Stanier (1984)	Nutrition and environmental temperature
Crenshaw *et al.* (1986)	Nutrition, temperature & age at weaning
Day and Webster (1999)	Early weaning
Dybkjaer (1992)	Behavioural indicators of stress at weaning
Fraser *et al.* (1994)	Diet quality and weaning
Friend *et al.* (1983)	Behaviour and weaning
Hampson (1986)	Digestive physiology
Keeling and Hurnik (1996)	Social facilitation
Kelly (1990)	Digestive physiology
Kelly *et al.* (2000)	Housing systems
Kenworthy (1976)	Digestive Physiology
McConnell *et al.* (1987)	Co-mingling & temperature
McGlone and Curtis (1985)	Aggression post-weaning
McKinnon *et al.* (1989)	Housing systems and Behaviour
Nabuurs *et al.* (1993)	Digestive physiology
Olesen *et al.* (1996)	Regrouping
Pajor *et al.* (1999)	Alternative housing systems
Pajor *et al.* (2002)	Alternative housing systems
Pluske and Williams (1996)	Split weaning
Pluske and Williams (1996)	Sedatives and co-mingling
Puppe *et al.* (1997)	Environment
Schrama *et al.* (1997)	Immune status
Thacker (1999)	Nutrition of early weaned piglets
Varley (1995)	Behaviour of newly weaned piglets
Waran and Broom (1992)	Creep feeding and weaning
Wattanakul (1997)	Grouping pre-weaning and post-weaning behaviour
Weary *et al.* (1999)	Pre-weaning environment
Whittemore and Green (2001)	Growth
Worobec *et al.* (1999)	Weaning age

1.8 AIM

The aim of this project was to assess the role of different stressors such as mixing unfamiliar piglets, relocation to a new environment and change in diet that are associated with weaning and how they affect piglet performance, immunity and behaviour. The research was carried out under conditions similar to a commercial enterprise to enable the findings to be applied by a producer without the significant cost of adapting buildings but by simply altering management practices.

The study was divided into three main areas that the literature highlighted to be key factors in the area of weaning. These factors were mixing with unfamiliar piglets, relocation to a new environment immediately post-weaning and creep feed availability. Following the initial study mixing piglets pre-weaning provided positive impact on piglet performance and behaviour therefore it was incorporated into further studies to assess any interactive effects between this and other factors.

CHAPTER 2. MATERIAL AND METHODS

2.1 EXPERIMENTAL ANIMALS AND HOUSING

High health (Enzootic Pneumonia negative and Porcine Reproductive and Respiratory Syndrome negative) PIC Camborough 15 (Large White x (Landrace x Duroc)) sows and their litters were housed at Harper Adams University College Pig Unit and farrowed in batches of 16 sows. However, the inability to bring on replacement gilts because of the foot and mouth crisis, during the last study (chapter 7) meant that it was necessary to use homebred gilts to enable the trial to continue. All treatments were balanced for parity and genotype throughout the experimental studies.

Seven days prior to farrowing sows were moved from the dry sow house into conventional farrowing crates and remained there until weaning. The farrowing pen (Figure 2.1) was $4.7m^2$ in size with a crate 1.1m high x 0.5m wide x 2.35m long and had a covered creep area with a triangular heatpad (82cm base x 110cm perpendicular).

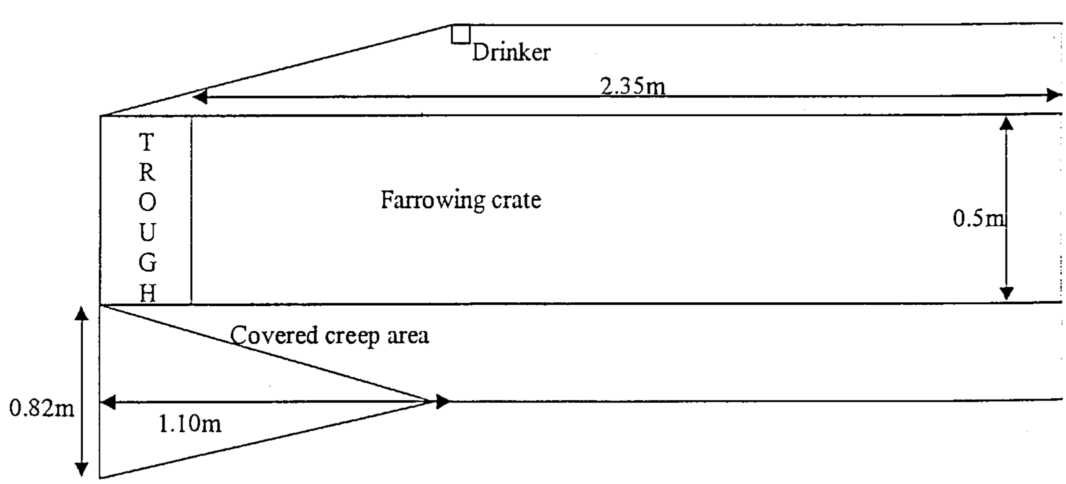

Figure 2.1 Diagram of conventional farrowing pen including crate and covered creep area (NB. Diagram not to scale)

Standard commercial procedures, such as teeth clipping, tail docking and administration of an iron injection intramuscularly (Gleptosil, Alstoe Animal Health Ltd, UK) giving 200mg/ml iron were carried out within 24 hours after birth. Each piglet was given an individual identification number tag and for behavioural observations were sprayed on their backs with a litter identification colour (Pig Marker) which remained apparent for 4-5 days and did not appear to affect their behaviour. Fostering of piglets was carried out between 24/48 hours only in the event of uneven litter sizes and within treatment group.

Weaning took place at 24 (chapter 6) or 28 days of age (chapters 3, 4, 5 and 7) when piglets were removed from the farrowing crates into one of three pen designs of fully slatted flat deck accommodation. In chapters 3, 4 and 6 each treatment group was housed in one pen (2.14m x 3.59m) with 3.59m of trough space divided into 18 feeding spaces and had 8 nipple drinkers available (Figure 2.2). In chapter 5 only two litters were housed together in the flat deck in pens (3.62m x 1.56m) with 2.14m of trough space divided into 12 feeding spaces and had access to 6 nipple drinkers (Figure 2.3). In chapter 7 new flat deck accommodation was built and each treatment group was again housed in one pen (1.55m x 5.16m) with 4.06m of trough space divided in to 16 feeding spaces and had 4 nipple drinkers available (Figure 2.4). Flat deck accommodation temperatures were maintained at 30°C for the first 7 days and then dropped 0.2°C per day for 5 days and then 0.5°C each day until 23°C was reached and maintained.

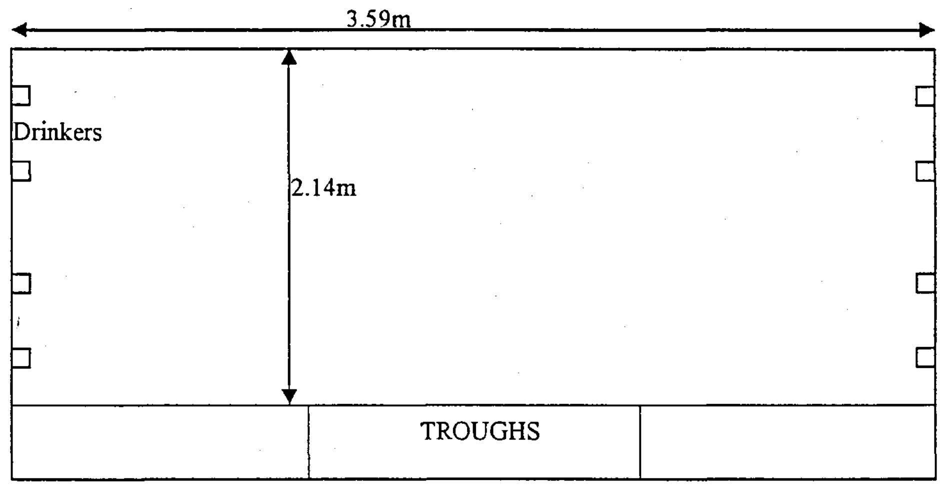

Figure 2.2 Diagram of weaner flat deck accommodation used in experimental chapters 3, 4 and 6 (NB. Diagram not to scale)

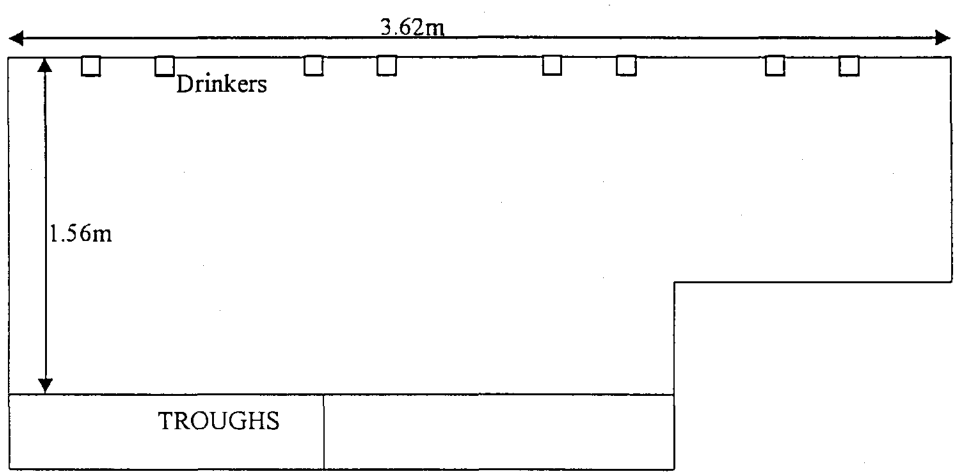

Figure 2.3 Diagram of weaner flat deck accommodation used in experimental chapter 5 (NB. Diagram not to scale)

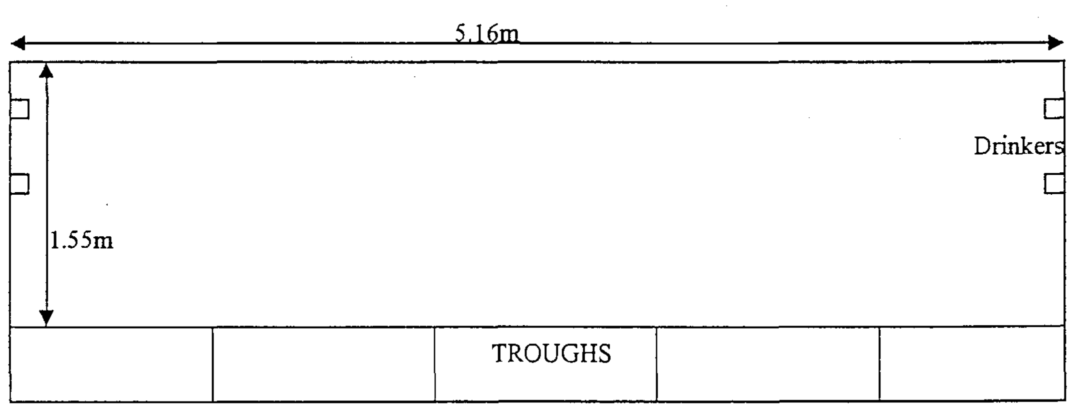

Figure 2.4 Diagram of weaner flat deck accommodation used in experimental chapter 7 (NB. Diagram not to scale)

2.2 DATA AND SAMPLE COLLECTION

2.2.1 Piglet live weights

Piglets were weighed at least once a week from birth to approximately 9 weeks of age and live weights recorded using a standard operating procedure (Appendix 1). A piglet trolley scales (Pharmweigh, Bury St Edmonds, UK) was used up to day 42 and Trutest Eziweigh 1 scales (Hanco Farm Supplies, Hertfordshire, UK) from day 43 onwards. Both scales were reading to 2 decimal places and accuracy checked using an appropriate range of standard weights.

2.2.2 Piglet lesion scores

At each weighing piglets were assessed for skin damage using a technique modified from de Koning (1983). The piglet's body was split into six areas – head, ears, shoulders, flank/rump, legs/feet and tail (Figure 2.5) and only fresh lesions were assessed. Each area was then assessed for the level of new skin damage using a scale from 0-4 as shown in Table 2.1.

Table 2.1 *Classification for lesion scores on each body area.*

Lesion Score	Classification
0	No Lesions
1	Low number (<5) of light lesions (scratches/no broken skin)
2	High number (>5) of light lesions
3	Low number (<5) of heavy/bleeding lesions
4	High number (>5) of heavy/bleeding lesions

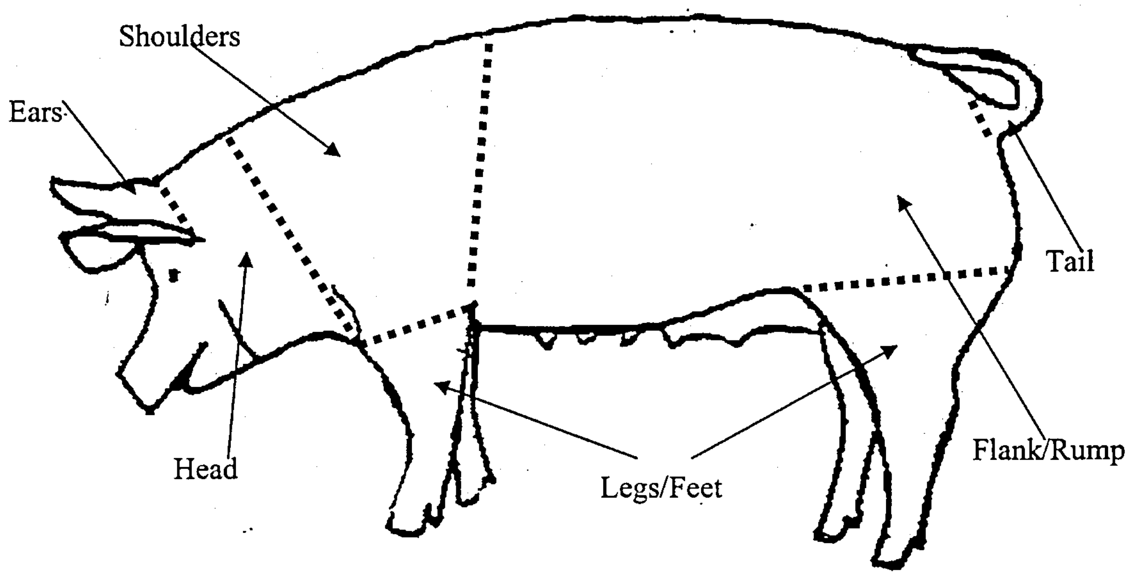

Figure 2.5 Diagrammatic representation of piglet's body divided into the six areas used to assess lesion score.

2.2.3 Blood collection

Blood samples were collected by venipuncture in the supine position into either 7ml non-additive vacutainers (BD vacutainer systems, Plymouth, UK) or 10ml lithium heparin vacutainers (BD vacutainer systems, Plymouth, UK) (Home Office Project Licence PPL 40/2109). Non-additive vacutainers were allowed to clot at 4°C overnight then centrifuged at 1000g for 15 minutes and the serum stored at –20°C until subsequent analysis.

2.2.4 Behavioural Observations

In section 3.2.4 a detailed behavioural study involving live behavioural observations was carried out to obtain a more detailed record of subtle behaviour patterns that may be missed by video recording (McGlone, 1986). Twenty four hour time-lapse video recording was used to assess more general behaviour patterns in section 7.2.5.

2.2.4.1 Pre-weaning behaviour observations

All observations were made starting at 07:00am and during each observational period the following was recorded: -

a) Number of sucklings,

b) Duration and time of sucklings,

c) Number of piglets sucking,

d) Proportion of alien piglets,

e) If the suckling was successful - A successful suckling was defined by the change in the sow's grunting rate combined with the rapid mouth movements of the piglets (Castrén *et al.*, 1989)

f) Sow or piglets initiated and terminated suckling - Initiation by the sow was defined as when the sow lay down and grunted to her piglets and initiation by the piglets was defined as when the piglets begin massaging the udder without previous grunting by the sow (Algers and Jensen, 1985). Termination was classified as when the sow rolled onto her belly or stood up, or when half of the litter had left or fallen asleep at the udder (Algers and Jensen, 1985).

g) Synchronised nursing was recorded if all three sows on the treatment began suckling within three minutes of each other (Wattanakul *et al.,* 1997b).

All agonistic interactions were noted and the following details recorded: -

a) Duration of fight

b) Littermates or non-littermate interaction.

c) Time fight occurred

A fight was defined as 'a series of five or more bites in a period of time lasting at least 15 seconds, and with no intervening periods longer than 60 seconds during which the piglets were separated (Rushen and Pajor, 1987).

A scan-sampling technique (Martin and Bateson, 1993) was used every ten minutes during each observational period to identify the general behaviour pattern of the piglets. Piglets were categorised into one of two mutually exclusive behaviours: -

a) Active – standing, feeding, walking, running

b) Lying

The number of piglets inside the creep was also noted every ten minutes.

2.2.4.2 Post-weaning behaviours

Observations were made for two hours immediately after weaning at 11:00am and then for two hours the following day. Another two hours of observations was carried out at 35 days of age. It is well documented that aggression post-weaning usually occurs in the first 90 minutes post-weaning (Friend *et al.*, 1983). Therefore, two-hour observational periods were used to allow all agonistic interactions to be recorded.

In the observational periods post-weaning, the same scan-sampling technique was used every ten minutes and piglets were categorised into one of the following mutually exclusive behaviours: -

a) Lying (Sternal or lateral recumbency)

b) Active –Not feeding

c) Feeding (Head in feed hopper)

d) Drinking

Again all agonistic interactions were recorded using the same technique as pre-weaning.

2.2.4.3 Teat orders

Teat order was assessed pre- and post-mixing. Each pair of teats was numbered with teat pair one being the most anterior teats and the piglet suckling those teats identified over two/three sucklings (Hessing *et al.*, 1994). The side the sow was laying on was also taken into consideration. Cross-suckling of the sows by the piglets was recorded during the teat order observation period post-mixing.

2.2.4.4 Creep feeding behaviour

Piglets were recorded for 24 hours using time-lapse video recorders (2 Sony SSCDC138P colour cameras, Sony SVT124P video recorder, Panasonic AG-6124 video recorder) in the final week prior to weaning to identify any feeding patterns and which piglets were eating the creep feed provided. Observations from these videos included duration of feeding, number of piglets feeding together and whether piglets were displaced from the feeder by another piglet.

2.2.4.5 Post-weaning choice feeding behaviour

Piglets were recorded for 24 hours using time-lapse video recorders (2 Sony SSCDC138P colour cameras, Sony SVT124P video recorder, Panasonic AG-6124 video recorder) to assess the effect of choice feeding and to identify any feeding behaviour patterns.

2.3 ANALYSIS OF DIETS

2.3.1 Feed samples

Commercial feeds (Ian Hollows Feed Supplements, Whitchurch, UK and Lloyds Animal Feeds, Wrexham, UK) were used throughout the trials to simulate a standard commercial situation. The main raw ingredients for each diet are shown in Table 2.2. Feed samples were collected and analysed for content of dry matter (DM), ash, crude protein (CP), ether extract (EE) and neutral detergent fibre (NDF) according to the method of MAFF (1993).

Table 2.2 *Main raw ingredients of each commercial diet fed (in descending by weight).*

Target 1	Target 2	Target 275	Target 3	Target 4	Lloyds Grower
Whey	Cooked wheat	Cooked wheat	Cooked wheat	Cooked wheat	Wheat
Cooked Maize	Cooked soya	Cooked soya	Cooked soya	Cooked soya	Barley
Cooked oats	Cooked oats	Cooked Maize	Cooked oats	Dehulled toasted extracted soya	Hipro soya
Full fat soya	Whey powder	Cooked oats	Cooked maize	Cooked barley	Maize Germ meal
Herring fishmeal	Cooked maize	Whey powder	Fishmeal	Wheatfeed	Fishmeal
Skimmed milk powder	Skimmed milk powder	Dehulled toasted extracted soya	Wheatfeed	Fishmeal	Wheatfeed

Feeding protocols varied slightly for each study depending on standard feeding practice on the farm to provide optimum growth and feed conversion ratio. Each feeding protocol is outlined within the following experimental chapters.

The feed declared specifications for each of the diets are provided in Table 2.3 and proximate analysis results can be found in each of the experimental chapters.

Table 2.3 *The declared feed specifications for all diets used during following studies.*

	Target 1	Target 2	Target 275	Target 3	Target 4	Grower
Chemical composition (declared specifications)						
Protein(g/kg)	245	230	220	220	215	220
Oil (g/kg)	135	95	70	70	60	60
Ash (g/kg)	65	65	65	65	60	47.5
Fibre (g/kg)	15	20	30	30	35	37.5
DE (MJ/kg)	18.0	16.5	15.5	15.5	14.75	-
Lysine (%)	1.8	1.65	1.45	1.55	1.45	1.40
Vitamin A (IU/kg)	15000	15000	15000	15000	15000	10000
Vitamin D3 (IU/kg)	2000	2000	2000	2000	2000	2000
Vitamin E (IU/kg)	250	250	150	150	150	60
Avilamycin (mg/kg)	40	40	40	40	40	-

2.3.2 Dry matter

Dry matter content (g/kg) was measured by weighing out approximately 1g of feed sample into a porcelain crucible and drying it in an oven at 80°C until it reaches a constant weight (usually overnight). The dry matter content (g/kg) was then calculated as:

$$\left\{\frac{\text{weight of dry sample (g)}}{\text{weight of original sample (g)}}\right\} \quad \text{x } 1000$$

2.3.3 Ash

Total ash content (g/kg DM) was analysed by weighing out approximately 1g of dried material into a porcelain crucible and then heated to 500°C in a muffle furnace

(Gallenkamp, size 3) for 16 hours. The remaining ash was cooled to room temperature in a dessicator and reweighed. Ash content (g/kg DM) was calculated as:

$$\left\{ \frac{\text{weight of ashed sample (g)}}{\text{weight of original sample (g)}} \right\} \times 1000$$

2.3.4 Crude protein

Crude protein (g/kg DM) was analysed by an automated Kjeldahl method using a Tecator 1035 autoanalyser (Foss UK Ltd, Oxon, UK). Samples of approximately 1g were weighed into digestion tubes containing two kjeltab catalyst tablets and then boiled in H_2SO_4 (6N, 14ml) at 400°C for 45 minutes. Digestion tubes were then allowed to cool before the addition of 75ml of distilled water. Nitrogen content was then determined by back titration using 0.2M HCl and crude protein content estimated (Total Nitrogen x 6.25)

2.3.5 Ether extract

Ether extract (g/kg DM) was determined using a Tecator soxtec 1043 extraction unit (Foss UK, Ltd, Oxon, UK). Approximately 2-3g of sample was weighed into a cellulose extraction thimble and then the thimble was plugged with de-fatted cotton wool. Samples were then boiled in 25ml of petroleum ether for 30 minutes and rinsed for 15 minutes. Any final traces of petroleum ether were evaporated off in a fume cupboard and fat content was calculated as:

$$\left\{ \frac{\text{weight of fat (g)}}{\text{weight of original sample (g)}} \right\} \times 1000$$

2.3.6 Neutral detergent fibre

Neutral detergent fibre content (g/kg DM) was determined by using the fibretec tecator 1020 (Foss UK Ltd, Oxon, UK). Approximately 0.5g of dried sample was weighed into a known weight glass crucible. The crucible was then placed in the fibretec apparatus and 25ml of cold neutral detergent fibre solution (93g disodium ethylene diamine tetra-acetate dihydrate (EDTA), 34 g of sodium borate, 150g of sodium lauryl sulphate, 50ml of 2-ethoxy ethanol mixed with 22.8g of anhydrous disodium hydrogen phosphate made up to 5 litres, pH adjusted to 6.9 - 7.1) and 0.5ml of anti-foaming agent (octanol) was added to the samples. Samples were boiled for 30 minutes and then 2ml of α-amylase (2g of α-amylase E.C.3.2.1.1 from *Bacillus subtilis* in 90ml of water filtered and 10ml of 2-ethoxy ethanol added and stored at 4°C) was added and the digestion procedure repeated. Samples were then filtered and washed three times using 20ml of hot distilled water. 25ml of hot water and 2ml of α-amylase was added and the samples left to stand for 15 minutes before being washed and then dried with 20ml of acetone. The crucible and digested sample was oven dried at 100°C overnight, cooled in a dessicator and weighed before being ashed at 550°C for 4 hours and then cooled and reweighed. Neutral detergent fibre content was calculated as:

$$\left\{\frac{\text{Residue weight (g) - weight of ashed sample (g)}}{\text{weight of original sample (g)}}\right\} \times 1000$$

2.4 IMMUNOLOGICAL METHODS

2.4.1 Preparation of keyhole limpet haemocyanin (KLH) antigen

KLH (Calbiochem, Novabiochem, UK) was dissolved in phosphate buffer saline, pH 7.2 (PBS) (Hudson and Hay, 1989) to give a 1mg/ml solution and sterilised by passage through a 0.2μm acrodisc syringe filter (PALL Gelman Laboratory, USA). The solution was then precipitated on alum and washed 3 times with PBS by repeated centrifugation and resuspension (Hudson and Hay, 1989). Finally the alum precipitated KLH was resuspended in PBS to make a 1mg/ml solution.

2.4.2 Determination of humoral immune response by Anti-KLH ELISA

Prior to analysis of test samples the optimum concentrations of the monoclonal antibodies (Mab) was assessed by 40% ammonium sulphate precipitation of porcine serum and serial dilutions of the Mab carried out. The optimum concentrations were identified as 1:200 dilution of serum and a 1:400 dilution for all Mab.

Anti-KLH antibody sub-classes (IgG_1, IgG_2, IgM and IgA) were determined by a direct ELISA method based on Pollock *et al.* (1991). Briefly, 96 well flat bottomed ELISA plates (Falcon mictrotitre plates, Becton Dickinson, France) were coated with 100μl of 10μg/ml KLH in PBS. The plates were incubated for 1 hour at 37°C followed by overnight at 4°C. Any excess KLH was removed by washing three times with PBS, then the remaining free sites of the wells were blocked with 150μl of 0.1% bovine serum albumin (BSA) (Sigma, Poole, UK) in PBS and incubated at 37°C for 2 hours. The plates were then washed three times with PBS containing 0.05% Tween 20 (Sigma, Poole, UK)

(PBS/T). Test sera was added to duplicate wells at the pre-determined optimal concentration of 1:200 in PBS/T and incubated at 37°C for 2 hours. After washing the plates three times with PBS/T, 100µl of mouse anti-porcine monoclonal antibodies (IgG_1, IgG_2, IgM, and IgA) (Serotec Ltd, Kidlington, Oxford, UK) at the pre-determined concentration of 1:400 in PBS/T and the plates incubated at 37°C for 1 hour. The plates were then washed three times with PBS/T and 100µl of 1:30000 dilution alkaline phosphatase conjugate (Sigma, Poole, UK) was added. The plates were incubated for 1 hour and then washed three times with PBS/T. Finally 100µl of 1mg/ml Sigma 104 Phosphatase substrate (Sigma, Poole, UK) dissolved in glycine buffer (pH 10.4) was added and the colour allowed to develop at room temperature in the dark. The plates were read on an ELISA plate reader (Benchmark microplate reader, Bio-Rad) at the optical density 405nm (OD_{405}) after 330 minutes.

2.4.3 Anti-soya ELISA

It has been reported that hypersensitivity to dietary soya antigens may be observed in piglets post-weaning (Miller *et al.*, 1994) therefore an anti-soya ELISA was also carried out to assess any possible dietary effect on immune response in chapter 4.

2.4.3.1 Purification of soya protein

Soya protein antigens were purified using the method by Miller *et al.* (1994). Defatted soya (soya bean meal) was oven dried and milled and 80g added to 800ml of Tris-HCl buffer (pH 7.8) containing 10mM-2-mercaptoethanol and stirred for 1 hour. The liquid was centrifuged at 10,000g for 30 minutes at 15°C and then the supernatant decanted off and the pH reduced to 4.8 using 5M HCl during continuous stirring and then stirred for a further 20 minutes. The liquid was centrifuged again at 10,000g for 30 minutes at 15°C and the precipitate resuspended in PBS. This resuspended precipitate was frozen at –80°C, freeze-dried and stored at 4°C in a dessicator.

2.4.3.2 Porcine Anti-soya ELISA

The soya antigen was prepared as in section 2.4.3.1 and then dissolved in carbonate-bicarbonate buffer (pH 9.6) to make a 10mg/ml solution. Ninety-six well flat bottomed ELISA plate (Falcon mictrotitre plates, Becton Dickinson, France) were coated with 100µl of 200mg/ml soya antigen and incubated at 37°C for 2 hours then overnight at 4°C. The plates were washed three times with PBS/Tween (PBS/T) and 100µl of 1:100 dilution of test serum in PBS/T was added before incubation at 37°C for 2 hours. The plates were washed three times in PBS/T and 100µl of 1:15000 dilution of anti-porcine IgG (alkaline Phosphatase conjugate, Sigma) in PBS/T then incubated for 1 hour at 37°C before being washed three times in PBS/T. Finally, 100µl of 1mg/ml Sigma 104 Phosphatase substrate in glycine buffer (pH 10.4) was added and the colour allowed to develop in the dark at room temperature. The plates were read on an ELISA plate reader (Benchmark microplate reader, Bio-Rad) at the optical density 405nm (OD_{405}) after 180 minutes.

2.4.4 Isolation of lymphocytes

Cell-mediated immune response was assessed using two lymphocyte blastogenesis assays (section 2.4.5), which required porcine peripheral blood mononuclear cells that were isolated by density gradient centrifugation.

Briefly, a 100% Stock solution of Percoll (Sigma, Poole, UK) was prepared by adding 1 part 1.5M PBS (x10 PBS) to 9 parts Percoll to get the percoll to the correct saline salt concentration, then a 62.5% solution was made by adding 6 parts PBS to 10 parts 100% percoll. A 62.5% concentration was used after a preliminary study determined that this was the optimum concentration for isolating the lymphocytes.

Blood samples were collected as previously described in section 2.2.3 in lithium heparin vacutainers. 7ml of blood was diluted with 7 ml of sterile PBS and then duplicate samples of 6ml of diluted blood was carefully layered onto 6ml of 62.5% percoll. The tubes were then centrifuged at 400g for 40 minutes at 4°C and the ring of cells at the interface between the percoll and the plasma collected. The collected cells were diluted with an equal volume of PBS and centrifuged at 350g for 7 minutes, then washed twice by resuspension and centrifugation.

Finally the cells were resuspended in 4ml of tissue culture medium (TCM) (Neonatal calf serum, sodium bicarbonate (20mM), L-Glutamine (2mM), Amphotericin B (3µg/ml), Gentamycin sulphate (50µg.ml) and RPMI 1640 (Sigma, Poole, UK)). Finally viable mononuclear cells were stained with 0.2% nigrosin and counted using an improved neubauer haemocytometer, then the number of viable cells calculated by:

$$\left\{ \frac{\text{number lymphocytes counted}}{\text{number triple-ruled squares}} \right\} \times 25 \times 10^4 \times \text{original dilution (if any)}$$

2.4.5 Lymphocyte transformation tests

Following the isolation of lymphocytes one of two techniques was used to assess cell-mediated immune response. These transformation or blastogenesis tests allow measurements of a cell-mediated immune response to be recorded. The radioisotope assay (chapter 4) has been widely used to assess lymphocyte proliferation (Whitbread, 1986; Becker and Misfeldt, 1993; Deguchi and Akuzawa, 1998), whereas the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (chapter 7) is a colorimetric assay thus removing the need for the use of [^{3}H] thymidine (Mosmann, 1983; Denzoit and Lang, 1986; Iwata and Inoue, 1993). However there have been a number of reports reporting a lack of correlation between the two different assays (Reubel and Bauerfeind, 1989).

2.4.5.1 Radioisotope assay

Once the lymphocytes had been counted, they were diluted to a concentration of 0.5×10^6 per ml, then serial dilutions carried out to provide cell suspensions of 0.25×10^6 and 0.125×10^6. Three dilutions were used to take into account the interaction between the uptake of tritiated thymidine and cell numbers and thus a mean was taken of the three cell concentrations used. Triplicate samples of each dilution were added to the wells of sterile

96 well round bottomed tissue culture plates with low evaporation lidS (Falcon microtitre plates, Becton Dickinson, France). Cells were stimulated with 20µl of either mitogen or control, the mitogen used was Concanavalin A (Con A) (Sigma, Poole, UK) (10µg/ml in RPMI 1640) and the control was RPMI 1640. The plates were then incubated at 37°C in 5% CO_2 for 48 hours. After incubation cells were labelled with 1µCi of ^{3}H Thymidine (TRA 306, Amersham) and incubated for a further 16 hours. Cell cultures were terminated by freezing the culture plates at –20°C and then defrosted prior to harvesting. Harvesting of cells was carried out using a Packard Filtermate 196 harvester onto 96 well filter plates (Unifilter-GF/C, Packard) by the addition of 25µl of Microscint-O (Packard) to each well before being counted on a scintillation counter (TopCount, Microplate Counter, Packard). Stimulation index was recorded by the mean of natural log counts per minute (CPM) for the 3 samples.

2.4.5.2 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay

Lymphocyte cell suspensions of 0.5 x 10^6 or 2 x 10^6 cells/ml were prepared as previously described in section 2.4.5. Sterile PBS was added to the outside wells of the 96 well tissue culture plates and 180µl of cell suspension was added in triplicate. Cells were stimulated with 20µl of one of the following mitogens, 5µg/ml Con A (Sigma, Poole, UK), 5µg/ml pokeweed mitogen (Sigma, Poole, UK) (PWM) or with 25µg/ml KLH and control wells with RPMI 1640. The plates were incubated at 37µC in 5% CO_2 for 48 hours then 20µl of 5mg/ml MTT (Sigma, Poole, UK) in PBS was added to each well and incubated for a further 4 hours at 37°C in 5% CO_2. Plates were centrifuged at 300g for 5 minutes and the supernatant aspirated off. 200µl of DMSO working solution (18ml

DMSO (Sigma, Poole, UK) and 2ml HCl (Sigma, Poole, UK)) was added and the plates left to stand for 15 minutes. The absorbance was read on an ELISA plate reader (Benchmark microplate reader, Bio-Rad) at an optical density of 570nm (OD_{570}) with a reference wavelength of 630nm (OD_{630}).

2.4.6 IFN–γ production assay

Interferon–gamma (IFN–γ) is a cytokine released by T helper cells that may create an environment that is indicative to type 1 immune responses and cellular immunity (Baldwin *et al.*, 2002). The IFN–γ assay kit (Tridelta Development Ltd, Ireland) is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) and is based on the release of IFN–γ from sensitised lymphocytes during an overnight incubation with a specific antigen (Walravens *et al.*, 2002).

Lymphocytes were isolated as previously described in section 2.4.3 and then diluted to a cell concentration of 2 x 10^6 cells/ml. 1ml of cell suspension was added to duplicate wells of a sterile 24 well microtitre plate (Falcon microtitre plates, Becton Dickinson, France) and 100μl of 5μg/ml Con A in RPMI 1640 was added. Plates were incubated at 37°C in 5% CO_2 for 16 hours and then centrifuged at 300g for 5 minutes. The supernatant was aspirated off and stored at –80°C.

IFN–γ was measured using the following method. Briefly, 100μl of IFN – γ standards (0, 7.8, 15, 31, 62, 125, 250 and 500pg/ml) was added to appropriate microtitre plate wells and then for all duplicate samples 50μl of standard diluent buffer containing 15mM sodium azide was added followed by 50μl of each sample. 50μl of biotinylated anti-IFN–γ (Biotin conjugate) was added to each well and the plate tapped gently to mix, followed by 2 hours incubation at 37°C. The plate was washed four times and then 100μl of Streptavidin-Peroxidase (HRP) working solution was added. The plate was incubated at room temperature for 30 minutes and washed four times. Finally 100μl of stabilised chromogen (Tetramethylbenzidine (TMB)) was added to each well and incubated for 30 minutes at room temperature. The absorbance was read using an ELISA plate reader (Benchmark microplate reader, Bio-Rad) at the optical density 450nm (OD_{450}). A regression of the standard concentrations and optical density readings was performed and IFN–γ concentrations (pg/ml) determined using the regression equation.

2.5 POST MORTEM PROCEDURE

Piglets were killed by an injection of sodium pentobarbitone. The abdomen was opened from the sternum to the pubis and the entire gastro-intestinal tract removed. The length and weight of the empty small intestine was measured and two sections (approximately 5cm in length) were removed at distances proportionately 25, 50 and 75% along the small intestine, then placed either in 3% Gluteraldehyde to fix them.

Adrenal glands of each pig were dissected away from the surrounding fat and weighed following evisceration. Adrenal weight was calculated as a proportion of final body weight of the piglet at slaughter.

2.5.1 Histology

After several days of fixation, the sections of small intestine was excised, dehydrated and embedded in paraffin wax. From each of these, three to five transverse sections (approximately 5μm) were cut and stained with haematoxylin and eosin before being examined under a light microscope. Measurements of villous height and crypt depth were taken from sections where the plane went vertically from tip of the villous to the base crypt. A calibrated eyepiece graticule was used to measure 10 villi and their associated crypts (Pluske *et al.*, 1997).

CHAPTER 3. THE EFFECT OF MIXING PIGLETS AT DIFFERENT AGES PRE-WEANING ON PIGLET PERFORMANCE, BEHAVIOUR AND IMMUNE FUNCTION

3.1 INTRODUCTION

The process of weaning is one of the most stressful periods in the piglet's life (Puppe *et al.*, 1997). Under natural conditions, social development and weaning is a gradual process, with no defined starting point but complete independence of the pig from the dam at approximately 17 weeks (Jensen and Recén, 1989). Commercially in the UK, weaning takes place abruptly between 3 and 5 weeks of age when piglets are usually mixed with non-litter mates and moved into a new, usually barren, environment (Puppe *et al.*, 1997). The effect of mixing, relocation and a change in diet occurring simultaneously causes great stress to the pig and a growth check is usually observed following weaning (Pluske and Williams, 1996b). This growth check is of significant importance to the producer as it causes a slowing in weight gain or a loss of weight (Okai *et al.*, 1976), therefore compromising the long-term performance of the pigs.

The mixing of non-litter mates is usually common practice in commercial situations in the UK yet it is one of the largest stressors occurring at weaning, leading to fights between unfamiliar individuals in order to determine the dominance hierarchy of the newly formed group (Ewbank, 1976; Petherick and Blackshaw, 1987). A number of methods have been used to reduce the stress of mixing at weaning such as varying group size (McConnell *et al.*, 1987) or varying weight ranges (Rushen, 1987), providing getaway barriers (Waran and Broom, 1993) or hides (McGlone and Curtis, 1985), administering tranquillisers such as amperozide/azaperone (Gonyou *et al.*, 1988, Barnett *et al.*, 1993; Pluske and Williams, 1996b), mixing into dimly lit accommodation (Christison, 1996) or at different times of the day (Ogunbameru *et al.*, 1992; Barnett *et al.*, 1994). All of these techniques have given varied results on post-weaning performance of piglets.

Another method that may be used to minimise the stress at weaning is to maintain groups at weaning either as individual litters (Musgrave *et al.*, 1991) or groups of litters mixed pre-weaning (Pluske and Williams, 1996b; Wattanakul *et al.*, 1997b). Grouping piglets prior to weaning may also increase the number of individuals that can be recognised therefore decreasing the number of unfamiliar pigs if piglets are to be mixed during the grower/finisher stages (Stookey and Gonyou, 1998). Wattanakul *et al.* (1997b) reported that agonistic behaviour did not significantly increase after grouping three litters together pre-weaning, at 11 days of age. Grouping prior to weaning also reduced levels of aggression observed after weaning at 28 days of age. Live weight and live weight gain were not affected by grouping prior to weaning and a trend in growth rate was seen after weaning with the previously grouped piglets tending to have higher daily live weight gains than the control (227g/g vs. 199g/d).

The aim of this study was to determine an optimum time at which mixing litters in a conventional farrowing crate system should occur to maximise performance of the piglet, without disrupting it's natural behavioural development but at the same time optimising it's welfare and that of the sow during the lactation period.

3.2 MATERIAL AND METHODS

3.2.1 Animals and Housing

Sixty PIC Camborough 15 (Large White x (Landrace x Duroc)) sows and their 677 piglets were kept in conventional farrowing crates (section 2.1) and randomly allocated to one of four different treatments. Five replicates were carried out and each replicate used 12 sows with three sows per treatment. Each treatment group was mixed at a different age: -

Treatment M7 – Mixed at ≈7 days of age (21 days before weaning)

Treatment M14 – Mixed at ≈14 days of age (14 days before weaning)

Treatment M21 – Mixed at ≈21 days of age (7 days before weaning)

Treatment M28 – Control (Not mixed until weaning on day 28)

Three litters were mixed together following work carried out by Blackshaw *et al.* (1987) that determined that it is better to mix piglets from three litters at weaning than two or four litters, therefore this principle was applied to mixing prior to weaning. Mixing of piglets was carried out by the removal of the boards between each pen so that the piglets from three litters could mix together whilst the sows were confined in the crate and for behavioural observations each litter was identified with a different coloured spray (Figure 3.1). Standard commercial procedures were carried out within 24 hours after birth as described in section 2.1.

Weaning took place at 28 days of age when piglets were removed from the farrowing crates into flat deck accommodation (section 2.1 and Figure 3.2) where each treatment group was housed in one pen. Split weaning, two days apart, was used to minimise age differences at weaning and enable behavioural observations to be carried out on all four treatments.

Figure 3.1 Piglets mixed at 14 days of age in a conventional farrowing room

Figure 3.2 Piglets mixed pre-weaning in the flat deck accommodation

3.2.2 Performance

Piglets were weighed after farrowing (day 0), then on days 7 (immediately before mixing treatment M7), 10 (3 days after mixing treatment M7), 14, 17, 21, 24, 28 (Day of weaning), then again on days 31, 35, 42 and 49 according to the standard operating procedure (section 2.2.1).

Creep feed (Target 2, Ian Hollows Feed Supplements) was supplied in one trough per litter and refreshed daily from day 14 onwards then weighed back at weaning. Post-weaning piglets were fed *ad libitum* on a three stage feeding regime starting with 3kg per piglet of Target 2 followed by 6kg per piglet of Target 3 and finally on to a grower feed (Lloyds Animal Feeds). Once the treatments total allocation had been eaten the next diet was supplied by mixing the remains of the previous diet to allow for an adjustment period. Dietary specifications can be found in Table 2.3 and proximate analysis was also carried out on the diets (Table 3.1) to check actual composition of the diets according to the methods outlined in section 2.3. There were Feed was weighed weekly to determine average feed intake per pig and feed conversion ratio (FCR).

Table 3.1 *Analysis of the creep feed and post-weaning diets provided.*

	Target 2	Target 3	Grower
Proximate analysis			
Dry Matter (g/kg)	897	902	876
Crude Protein (g/kg DM)	237	256	220
Oil (g/kg DM)	60	96	22
Ash (g/kg DM)	69	64	52
NDF (g/kg DM)	134	117	163
DE (MJ/kg DM)†	16.1	17.3	15.6
Vitamin E (IU/kg)	250	150	60
Avilamycin (mg/kg)	40	40	-

† DE = 17.47 + 0.0079CP + 0.0158Oil – 0.0331Ash – 0.0140NDF
(CP, Oil, Ash and NDF in g/kg DM) based on calculation by MAFF (1993)

3.2.3 Lesion Scoring

Each piglet was examined at each weighing for lesions using the method described in section 2.2.2. The classifications used are shown in Table 2.1.

Lesion scores for each body part were totalled up to give a total body score and the head, shoulders and ears were also totalled to give a front body score. This gave an indication of the amount of fighting carried out over each period, as the majority of lesions on these areas resulted from aggressive interaction with other piglets. There were negligible lesions observed on the flank and tail so the data was excluded from further analysis.

3.2.4 Behavioural Observations

The behaviour study carried out aimed to assess if mixing piglets at different developmental stages caused any differences in behaviour. Live behavioural observations were carried out to obtain a more detailed record of subtle behaviour patterns that may have been missed by video recording (McGlone, 1986).

3.2.4.1 Pre-weaning behaviour

For each treatment group, observations were made for three hours prior to mixing and six hours immediately post-mixing. Another three hours of observations were made on the day following mixing and three days later. The control group (treatment M28) was observed for three hours prior to weaning. All observations were made at the same time of day for each treatment. Full details of the behaviours observed are in section 2.2.4.1.

During each observational period the following was recorded: -

a) Number of sucklings

b) Duration and time of sucklings

c) Number of piglets suckling

d) Proportion of alien piglets at suckling

e) If the suckling was successful

f) Sow or piglets initiated and terminated suckling

g) Synchronised nursing

All agonistic interactions were noted and the following details recorded: -

a) Duration of fight

b) Littermate or non-littermate interaction.

c) Time fight occurred

Piglets were also categorised into one of two mutually exclusive behaviours using a scan sampling technique: -

a) Active – standing, feeding, walking, running

b) Lying

The number of piglets inside the creep was also noted every ten minutes.

3.2.4.2 Post-weaning behaviour

All the treatment groups were observed for two hours immediately after weaning and then for two hours the following day at 29 days of age. Another two hours of observations were carried out at 35 days of age. It is well documented that aggression post-weaning usually occurs in the first 90 minutes post-weaning (Friend *et al.*, 1983). Therefore, two-hour observational periods were used to allow all agonistic interactions to be recorded. In the observational periods post-weaning, the same scan-sampling technique was used every ten minutes and piglets were categorised into one of the following mutually exclusive behaviours: -

a) Lying (Sternal or lateral recumbency)

b) Active –Not feeding

c) Feeding (Head in feed hopper)

d) Drinking

Again all agonistic interactions were recorded using the same technique as described in section 2.2.4.2.

3.2.5 Statistical analysis

Statistical analysis was performed using Genstat for Windows (Version 4.1). As there is a sequential nature to weight gain live weight data were analysed using Antedependence Modelling to determine if there was an effect of treatment over time (Kenward, 1987). It has been shown that univariate ANOVA cannot be used for repeated measures data due to the dependence of the data over time so one method for handling this data was to model this dependence and then adapt ANOVA to take the dependence into account (Kenward, 1987). There are two stages to antedependence modelling, First to determine the order of the model and secondly to construct the ANOVA accordingly. Analysis of variance was performed on all other performance data along with regression analysis for daily live weight gain. Treatment means were compared using a Protected Least Significant Difference (LSD) (Snedecor and Cochran, 1993). Behavioural data were analysed by Chi-square (χ^2) tests. Statistical significance was accepted at $P<0.05$, however it is pertinent to discuss any possible trends that were observed which had a statistical significance between 0.05 and 0.10

especially in terms of the behaviour measurements where $P<0.10$ is the accepted level of significance (Morris, 1999). In the farrowing house, the sow and her litter were identified as the experimental unit (d.f. = 52) and in the flat deck the whole pen was used as the experimental unit (d.f. = 12).

3.3 RESULTS

3.3.1 Performance

Mortality rates that occurred during the pre-weaning period averaged at 4% and were equal across all treatments. Several smaller piglets had to be removed from the flat decks post-weaning (4%). However, these piglets were observed in all treatments and therefore the age at mixing had no significant effect on the number of small piglets.

The order two antedependence model of live weight (Table 3.2) shows that there was an effect of treatment over the trial period but only at certain stages. Piglets mixed at 7 days had a significantly lower weight compared with the remaining non-mixed treatments ($P<0.001$). By day 24, there was a tendency for piglets mixed pre-weaning on day 7, 14 and 21 to have a lower live weight compared with the unmixed treatment M28 ($P=0.068$).

In the period immediately post-weaning there was no significant effect of mixing pre-weaning on live weight. By day 35, there was a tendency for piglets mixed at 21 days of age to have a lower live weight compared to the other three treatments and by day 42 this difference had become significant ($P=0.045$). Piglets mixed at 14 days had a significantly higher live weight compared with the other three treatments by day 49 and piglets mixed at 7 days of age had a significantly lower live weight compared to the piglets mixed at 21 days and at weaning ($P<0.001$).

Table 3.2 *Effect of mixing at different ages prior to weaning on piglet live weight*

	Mixed at (age) 7 days (M7)	14 days (M14)	21 days (M21)	Weaning (M28)	s.e.d	Significance
Live weight (kg)†						
Birth	1.46	1.48	1.49	1.51	0.072	NS
Day 7	2.77	2.67	2.64	2.70	0.119	NS
Day 10	3.46	3.45	3.49	3.50	0.057	NS
Day 14	4.52	4.63	4.50	4.53	0.068	NS
Day 17	5.27	5.41	5.42	5.47	0.041	<0.001
Day 21	6.46	6.53	6.44	6.53	0.059	NS
Day 24	7.35	7.33	7.32	7.45	0.057	0.068
Day 28(wean)	8.32	8.42	8.31	8.38	0.115	NS
Day 31	8.70	8.71	8.74	8.58	0.094	NS
Day 35	9.66	9.40	9.38	9.50	0.116	0.066
Day 42	12.29	12.13	11.76	11.98	0.187	0.045
Day 49	14.98	16.01	15.49	15.48	0.187	<0.001

† Means adjusted for the previous two weights as covariates for Antedependence model order 2

Piglets mixed at 7 days of age had a significantly lower daily live weight gain compared to the other treatments from days 10-14, 14-17 and 17-21 (P=0.024; P=0.001 and P=0.006 respectively). There appeared to be no immediate effect of mixing at 14 days on daily live weight gain post-mixing (Table 3.3) yet from days 21-24 all of the mixed treatments had a significantly lower daily live weight gain compared to the unmixed treatment (P=0.003). Piglets mixed at 7 and 21 days of age tended to have lower daily live weight gains compared to piglets mixed at either 14 days or at weaning (P=0.093).

During the post-weaning period there was no immediate effect of mixing prior to weaning as all treatments experienced a similar reduction in daily live weight gain in the first three days after weaning (Table 3.3). However, it can be seen that a longer-term effect emerged in the post-weaning period. Piglets mixed at 7 days were growing significantly faster compared to piglets mixed at 14 and 21 days of age from day 31 to 35 (P=0.030). Then from day 35 to 42, piglets mixed at day 7 were gaining significantly more weight compared to piglets mixed at 21 days of age and at weaning (P=0.008). From day 42 onwards, piglets mixed at 14 days of age had a significantly higher daily live weight gain compared with the other three treatments (P<0.001).

From weaning to day 49 piglets mixed at 7 and 14 days had significantly greater daily live weight gains compared to the other two treatments groups (P=0.047). Over the entire trial period, from birth to day 49 piglets mixed at 14 days had significantly higher growth rates compared with piglets mixed at 7 and 21 days. Also piglets mixed at weaning had a significantly greater daily live weight gain compared to treatment M21 (P=0.020).

Table 3.3 *Effect of mixing at different ages on pre- and post-weaning daily live weight gain*

	Mixed at (age) 7 days (M7)	14 days (M14)	21 days (M21)	Weaning (M28)	s.e.d	Significance
DLWG (g/day)						
Birth – day 7†	185	190	184	175	14.8	NS
Day 7-10†	200	193	201	208	19.7	NS
Day 10-14†	241	284	242	267	15.8	0.024
Day 14-17†	241	298	286	309	17.1	0.001
Day 17-21†	241	276	256	285	13.0	0.006
Day 21-24†	239	244	231	287	15.3	0.003
Day 24-W†	239	271	236	266	17.1	0.093
W - day 31‡	116	120	98	86	32.7	NS
Day 31-35‡	236	160	166	184	27.2	0.030
Day 35-42‡	414	368	317	358	27.0	0.008
Day 42-49‡	421	567	490	486	25.6	<0.001
Birth – W†	238	258	243	263	10.1	0.056
W- day 49‡	342	363	317	331	16.6	0.047
Birth – day 49†	281	303	275	296	9.6	0.020

† Means adjusted for birth weight as a covariate ‡ Means adjusted for weaning weight as a covariate

Daily live weight gain was also analysed using regression analysis over weekly period and similar trends are apparent although these are not significant pre-weaning (Table

3.4). Post-weaning from day 42 to 49, piglets mixed at 14 days had significantly higher daily live weight gains compared to piglets mixed at 7, 21 and 28 days (418 vs. 569 vs. 486 vs. 497 for treatments M7, M14, M21 and M28 respectively, P< 0.001).

Table 3.4 *Daily live weight gains for piglets mixed at four different ages pre-weaning (regression analysis)*

	Mixed at (age)				
	7 days (M7)	14 days (M14)	21 days (M21)	Weaning (M28)	Significance
DLWG (g/day)					
days 0-7	183	170	165	176	NS
days 7-14	259	271	257	269	NS
days 14-21	244	293	271	303	NS
days 21-28	258	272	252	287	NS
days 28-35	190	151	156	149	NS
days 35-42	406	376	311	367	0.044
days 42-49	418	569	486	497	<0.001
days 0-49	272	285	260	282	NS

Due to some problem with the environmental controls of the flat deck accommodation during replicate three there was a high level of wastage that was not accounted for. Therefore Table 3.5 gives the feed intakes and feed conversion ratios minus the data from replicate three. However, this did not appear to affect any of the other results directly.

From weaning to day 42, there was no difference in the piglets feed conversion ratios (FCR). However, piglets mixed at 7 days had a significantly greater FCR compared to the other three treatments from day 42 to 49 (P=0.025). Piglets mixed at 7 days tended to consume more feed, compared with piglets mixed at 21 days and weaning, in the period from weaning to day 35 (P=0.060). Piglets mixed at 14 days of age had a significantly higher average feed intake from day 42 to 49 compared with piglets mixed at 21 and 28 days (weaning) and also piglets mixed at day 7 consumed significantly more than piglets mixed at 21 days of age (P=0.029). A similar trend was also observed over the entire post-weaning period (P=0.056).

Table 3.5 *Effect of mixing at different ages pre-weaning on Average Feed Intake and Feed Conversion Ratios (FCR) during the post-weaning period*

	Mixed at (age) 7 days (M7)	14 days (M14)	21 days (M21)	Weaning (M28)	s.e.d	Significance
Average Food Intake (g/pig/day)						
W – day 35	284	257	205	232	25.4	0.060
Days 35-42	439	423	362	404	36.8	NS
Days 42-49	630	651	534	575	34.3	0.029
W – day49	524	547	464	484	27.9	0.056
FCR						
W – day 35	1.26	1.56	1.62	2.04	0.449	NS
Days 35-42	1.14	1.27	1.21	1.21	0.101	NS
Days 42-49	1.58	1.27	1.20	1.30	0.104	0.025

3.3.2 Lesion scores

Total body lesion scores (Figure 3.3) increased for each treatment after the first three days post-mixing. This pattern follows for the three treatments mixed pre-weaning but when looking at piglets mixed at weaning there was a significant increase in the lesions and this significantly higher level was still apparent 7 days post-weaning. Front body lesion scores followed a similar pattern to total lesion scores.

It can be noted that as the piglets get older the severity and number of lesions increased. There was a rise in lesion score at weaning for all treatments as indicated in the lesion scores on day 31. This may indicate that weaning still caused some increase in aggression, even between the piglets that had been mixed previously.

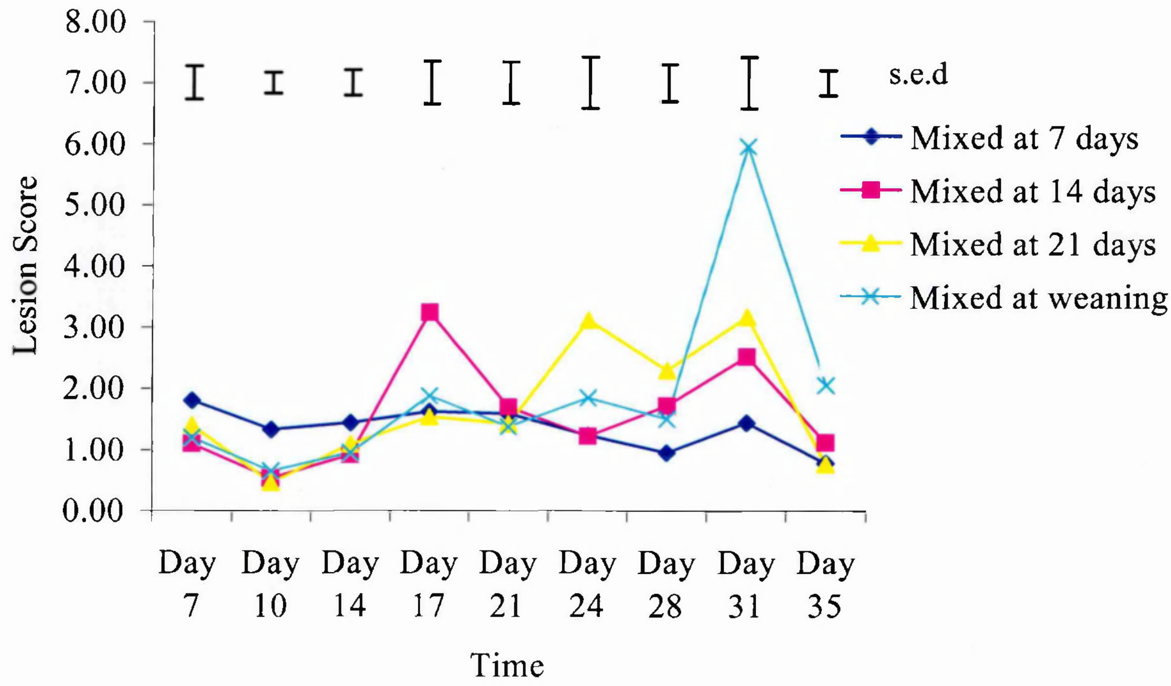

Figure 3.3 Effect of mixing at different ages prior to weaning on piglet total body lesion score

3.3.3 Pre-weaning behaviour

No significant effect of mixing at any age was seen on general activity or on the amount of time piglets spent in the creep area prior to weaning. Agonistic interactions between piglets were recorded on all observation days and the average number of fights during each hour (Figure 3.4) revealed no significant difference between treatments on the day prior to mixing or on the study days post-mixing prior to weaning. Although in the first hour immediately after mixing there was a tendency for piglets mixed at 7 days to have fewer fights compared with piglets mixed at 14 and 21 days (P=0.068).

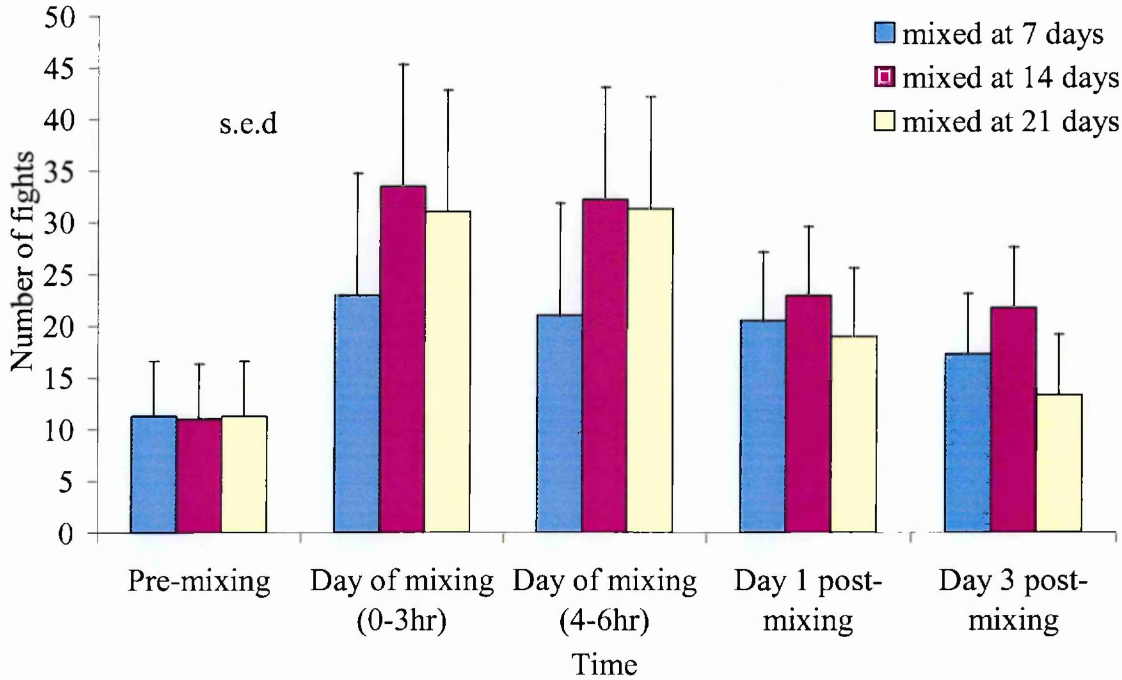

Figure 3.4 Effect of mixing pre-weaning on average number of fights in each hour of the pre-weaning observation days around the mixing process

There was no significant effect of treatment on the number of fights lasting longer than 1 minute when comparing the three treatments mixed prior to weaning during any of the observational periods. The number of fights observed between non-littermates or littermates was not significantly affected by the age of mixing prior to weaning.

Piglets mixed at 21 days had significantly more fights over teats compared with piglets mixed at day 7 or day 14 (0.831 vs. 0.492 vs. 0.486 respectively, χ^2= 9.701). There was no other significant effect of mixing prior to weaning on the proportion of fights over teats at any other time.

3.3.3.1 Suckling behaviour

To allow the analysis of suckling behaviour between and within treatments the proportional change from pre-mixing to the observational periods post-mixing were calculated. There was no significant treatment effect on the number and duration of sucking and suckling interval pre-mixing (day –1) or in the proportional changes (Table 3.6).

No significant effect of treatment on the number of successful sucklings, sucklings initiated or terminated by the sow, synchronised sucklings or number of cross sucklers were observed on any of the behavioural study days.

Table 3.6 *Effect of mixing at different ages on the average number, duration of sucklings and suckling intervals on day –1 (pre-mixing) and the relative change from pre-mixing to post-mixing*

	Mixed at (age) 7 days (M7)	14 days (M14)	21 days (M21)	s.e.d	Significance
Number of successful sucklings					
Day –1	4.3	4.2	4.0	0.30	NS
Change in number of sucklings (%)					
Day –1 vs. Day 0	6.7	10.5	4.0	9.75	NS
Day –1 vs. Day 1	-3.9	3.7	-14.6	9.80	NS
Day –1 vs. Day 3	8.9	1.8	-7.4	8.19	NS
Duration of complete sucklings (secs)					
Day –1	361	296	297	39.5	NS
Change in duration of sucklings (%)					
Day –1 vs. Day 0	-22.1	-12.5	-14.6	9.56	NS
Day –1 vs. Day 1	8.6	14.0	-16.4	15.34	NS
Day –1 vs. Day 3	-8.3	-3.3	-2.8	12.80	NS
Suckling Interval (secs)					
Day –1	2368	2318	2712	335.5	NS
Change in suckling interval (%)					
Day –1 vs. Day 0	8.5	2.7	-1.1	11.61	NS
Day –1 vs. Day 1	23.0	-2.0	7.0	27.60	NS
Day –1 vs. Day 3	19.0	-7.2	2.9	19.86	NS

3.3.4 Post-weaning behaviour

Figure 3.5 shows the number of fights between piglets immediately after weaning and on days one and seven post-weaning. It is clear that piglets mixed at weaning fought significantly more immediately post-weaning compared to the three treatments mixed prior to weaning (P=0.001). However, the first hour post-weaning was the only time point where there was a significant effect of treatment on the number of agonistic interactions observed.

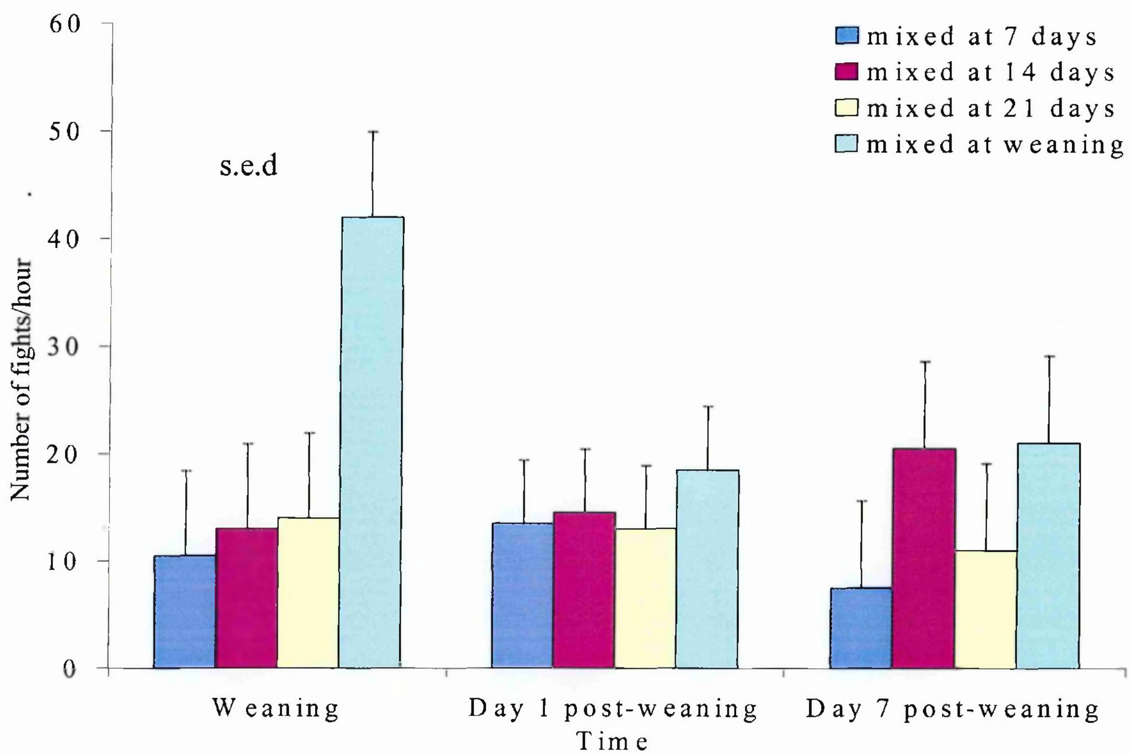

Figure 3.5 Effect of mixing at different ages prior to weaning on the average number of fights per hour on the observational days post-weaning

There was no significant effect of treatment in the post-weaning period on the proportion of fights between non-littermates. Table 3.7 allows comparison between the first hour post-weaning and the first hour post-mixing. Piglets mixed at weaning had more fights lasting longer than piglets mixed prior to weaning in the first hour post-weaning (P<0.001).

Table 3.7 *The proportion of fights in the first hour post-mixing and the first hour after weaning between littermates or non-littermates and over 60secs.*

	Mixed at (age) 7 days (M7)	14 days (M14)	21 days (M21)	Weaning (M28)	χ^2	Significance
At Mixing						
Prop. Fights >60s	0.042	0.074	0.188	-	48.724	NS
Prop. Fights Non-littermates	0.577	0.648	0.696	-	3.7996	NS
At Weaning						
Prop. Fights >60s	0.148	0.184	0.337	0.576	16.930	<0.001
Prop. Fights Non-littermates	0.574	0.724	0.853	0.777	4.2329	NS

3.4 DISCUSSION

3.4.1 Performance

The performance data from this study was broadly consistent with previous studies where mixing pre-weaning at a single age has seen a reduction in aggression (Pluske and Williams, 1996b) and occasionally trends for improvements in growth rates post-weaning (Wattanakul *et al.*, 1997b). The lower live weight observed in the piglets mixed at 7 days of age may have been caused by the disruption to the teat order too soon after initial formation in the first week post-farrowing as previously reported by Horrell and Bennett (1981). Fostering 1-week-old piglets resulted in the whole suckling process being disturbed and the fighting and squealing of the piglets caused the sow to be very restless and often terminate suckling prior to milk letdown thus reducing growth rates. The decrease in growth rate observed may also be attributed to piglets being absent from suckling periods due to lack of communication from sow or confusion by the piglets as reported by Pedersen *et al.* (1998) where it was identified that piglets which missed a suckling on their own sow rarely suckled on a foreign sow. This is supported by the behavioural data collected (section 3.4.3) where a very low incidence of cross-suckling was observed.

Piglets mixed prior to weaning still had a growth check immediately post-weaning although piglets mixed earlier maintained a slightly higher daily liveweight gain compared to piglets mixed closer to weaning. Wattanakul *et al.* (1997b) observed a similar effect of mixing pre-weaning; with the treatment mixed at 11 days having a slightly higher growth rate than piglets mixed at weaning. Piglets allowed to co-mingle from 10 days of age were significantly heavier ($P<0.05$) at weaning and remained heavier through to 14 days post-weaning (Pluske and Williams, 1996b).

Creep and water intake were not affected by mixing although there was a tendency for the piglets mixed prior to weaning to consume more creep feed than the control. This is possibly the result of social facilitation, a behavioural phenomenon of synchronised feeding and the fact that eating is often socially facilitated (Keeling and Hurnik 1996). Therefore the presence of more piglets encouraged others to feed as well.

As stated in section 3.3.1 a number of piglets were removed from the flat decks during the post-weaning period due to poorer growth rates and therefore they were disadvantaged in the large pen size. Bearing this in mind it is important to remember that these piglets will require other accommodation and thus additional weaner accommodation may be necessary to provide the best husbandry for these smaller piglets.

3.4.2 Lesion Scores

de Koning (1983) identified that changes in behaviour and health status will show up as changes in lesion patterns in sows and the state of the skin of an individual reflects their well-being. Therefore, the reduction in lesions observed by mixing pre-weaning may indicate improved health and well-being. This use of lesion scoring may be a useful method of assessing aggression and possibly assessing overall welfare/well-being within a group of piglets.

Lesion scores increased after each mixing during lactation and the results show that the earlier in life that the piglets are mixed the less severe their lesions. Pitts *et al.* (2000) found similar results when testing pairs of unfamiliar individuals at various ages from 5-26 days and suggested that two factors might explain why younger piglets did less damage whilst fighting: a) younger piglets spent less time fighting and b) they were physically smaller and therefore could not inflict large visible wounds. These two factors may explain the effect of mixing earlier pre-weaning reduces lesion score when compared to mixing at weaning.

The observation that at weaning aggression was increased in all groups of piglets is supported by the work of Puppe *et al.* (1997) who stated that newly weaned piglets appear to have more problems coping with a new environment than coping with unfamiliar piglets. Lesion scores three days post-weaning were significantly greater in piglets mixed at weaning and skin damage scores post-weaning did not increase significantly in treatments mixed prior to weaning supports similar results found by Wattanakul *et al.* (1997b) when assessing mixing piglets prior to weaning at 11 days of age.

Mixing at weaning also caused the piglets to continue fighting for longer compared to the time it took for fighting to subside when piglets were mixed pre-weaning as indicated by the high level of lesion scores seven days after weaning (Figure 3.3). After mixing pre-weaning the incidence of fighting had reduced to similar levels after three days as also reported by Wattanakul *et al.* (1997b).

Stookey and Gonyou (1998) showed that piglets fought more with unfamiliar pigs compared to pigs that they recognised from previous experience when paired at weaning (day 28) with siblings or non-siblings which had either been reared together or separately. As the piglets mixed pre-weaning in this study also had lower lesion scores post-weaning and fewer fights occurred after weaning when piglets remained in their treatment groups it would indicate that these piglets were able to recognise each other so less aggression was needed to maintain the dominance hierarchy (Rasmussen *et al.*, 1962; Symoens and Van Den Braude 1969).

It may be possible to use the assessment of fresh lesions during periods of change as a measurement for the levels of aggression and the time taken for aggression levels and incidence of fighting to reduce. If lesion scores are indicative of welfare (de Koning, 1983), then welfare will be improved with lower lesion scores.

3.4.3 Pre-weaning behaviour

In this study, general behaviour patterns pre-weaning were not affected by mixing indicating that there were potentially no detrimental effects or unnecessary stress associated with mixing prior to weaning.

The agonistic interactions of the piglets pre-weaning were not significantly affected by treatment but all three treatments mixed prior to weaning showed an increase in the number of fights observed corresponding with the increase in lesion scores observed. The formation of teat orders and social dominance hierarchies becomes stable after approximately 48 hours (Scheel *et al.*, 1977; Graves, 1984) and this is likely to be the same for the formation of new social hierarchies after mixing pre-weaning as agonistic interactions decreased in number after the initial day of mixing.

When the number of fights between littermates was compared with the number of fights between non-littermates no significant difference was seen. This may indicate that the piglets were unable to identify members of their own litter or needed to re-establish dominance. Conversely, Stookey and Gonyou (1998) reported that piglets reared together, regardless of genetic relatedness, fought less when regrouped after weaning compared with unfamiliar piglets demonstrating that piglets can recognise each other through mutual association. However, it may not be the recognition of other individuals that causes the aggression but possibly that weaning causes a disruption in the recognition of the subtle behaviours that are required to maintain the dominance hierarchy as suggested by Rasmussen *et al.* (1962).

3.4.3.1 Suckling behaviour

As the age of the piglets increases and milk yield of the sow decreases the number of sucklings reduces in semi-natural conditions (Jensen, 1988; Newberry and Wood-Gush, 1988) and this supports the slight reduction in the number and duration of sucklings observed between treatments prior to mixing.

Wattanakul *et al.* (1997b) found that cross-suckling and foreign piglets disrupted the suckling pattern and more suckling attempts were made as supported by Olsen *et al.* (1998). All three sows within each treatment suckled in synchrony allowing all of the piglets to suckle at the same time and thus avoiding foreign piglets disrupting the suckling of other sows. Previously fostering of piglets at 1 week of age has been seen to disrupt suckling by squealing and fighting of piglets at the udder, in turn causing the sow to become restless and terminate sucklings prior to milk letdown (Horrell and Bennett, 1981).

Horrell (1982) increased number of initiated sucklings and a significant decrease in actual milk letdowns along with increased intervals between milk letdowns when fostering 1 week old piglets lead to a reduction in growth rate of piglets. This may have been mirrored in the growth rates of piglets mixed pre-weaning compared to the control piglets remaining as entire litters. The limited effects of mixing pre-weaning on suckling patterns may be interpreted as a positive result as it indicates that there are no detrimental effects on the behaviour of the sow or piglets due to mixing prior to weaning.

3.4.4 Post-weaning behaviour

Initially, piglets are likely to spend time investigating their new environment and it has been reported in previous studies after weaning (28 days) that the initial exploratory behaviour was directed towards the environment and that fighting appeared to commence once the novelty of the environment wore off (Friend *et al.*, 1983). Within the two-hour observational period all the piglets had settled down and were lying together in all treatment groups after a short period of intense exploratory behaviour.

Immediately post-weaning, piglets mixed at weaning were observed fighting significantly more in the first compared to the three treatments mixed pre-weaning. The increased aggression corresponds to the high levels of lesions observed three days post-weaning as noted in previous reports by Blackshaw *et al.*, (1987); Worobec *et al.*, (1999); Pitts *et al.*, (2000).

Pitts *et al.* (2000) showed that the piglets benefited in terms of reduced aggression at weaning, after mixing prior to weaning, by placing pairs of piglets at various ages from 5 to 26 days old in a pen together and suggested that this would have potential welfare advantages in their long-term performance. Minimising aggression between piglets has been shown to improve their welfare and production. Any method of reducing aggression at weaning can have a positive effect on the future welfare and performance of the piglet (Petherick and Blackshaw, 1987) and, therefore, mixing piglets earlier in their development as carried out in this study may be of practical benefit in commercial pig production.

3.5 CONCLUSIONS

Overall it can be concluded that mixing pre-weaning does not detrimentally affect growth rates and that, if mixed at 14 days, an increase in performance may be observed. Lesion scores were significantly reduced the earlier that piglets were mixed prior to weaning, possibly resulting in improved piglet welfare.

Mixing piglets prior to weaning by removing the boards between sows is an inexpensive method of reducing post-weaning aggression and allowing the piglets to integrate with other piglets at an age when the development of social relationships occurs in semi-natural conditions. There were no apparent detrimental effects on the behavioural patterns of the piglets as a result of mixing, at any time pre-weaning. There was a clear trend for improved performance and reduced lesion score levels when mixing occurred at 14 days of age.

CHAPTER 4. THE EFFECT OF MIXING PIGLETS PRE-WEANING ON PIGLET IMMUNE FUNCTION

4.1 INTRODUCTION

The immune system of piglets weaned commercially (i.e. between 21-28 days of age) is not fully functional because of the drop of maternal antibodies circulating in the milk supply at a time when the piglet's own immune system is still underdeveloped. It is known that piglets weaned between 21-28 days of age are more susceptible to infections because of the rapid reduction in maternal immunity (Smith, 1992). It has also been shown that the stress of weaning can cause changes to the immune system (Kelley, 1982) and reduces its ability to control disease (Haye and Kornegay, 1979; Blecha *et al.*, 1983). Both humoral (Blecha and Kelley, 1981) and cell-mediated immune responses (Blecha *et al.*, 1983) can be adversely effected. If weaning has altered the piglet's immune response and thereby reduced it's ability to produce antibodies (Blecha and Kelley, 1981) then the timing of vaccination programmes (e.g. enzootic pneumonia) at weaning may be inappropriate.

Transient hypersensitivity is well documented in the early-weaned piglet (Li *et al.*, 1990; Miller *et al.*, 1991; Dréau *et al.*, 1994) and has generally been caused by hypersensitivity reactions to soya proteins within the diet leading to disturbances in the digestive process and resulted in decreased growth performance (Hankins *et al.*, 1992). Knowledge of the relationship between the humoral immune response and soya proteins has the potential to prevent post-weaning digestive disturbances (Hankins *et al.*, 1992).

In Chapter 3, the optimum time of mixing pre-weaning in terms of reducing aggression and improving growth performance was shown to be 14 days of age. If by separating one of the stressors from the point of weaning (i.e. mixing of unfamiliar piglets) the stress at weaning may be reduced therefore allowing the immune system to cope with any infection. Hence, the aim of this study was to determine if mixing piglets at 14 days of age, prior to weaning, affects the piglet's humoral and cell-mediated immune responses post-weaning compared with conventionally weaned piglets.

4.2 MATERIAL AND METHODS

4.2.1 Animals and Housing

Twelve PIC Camborough 15 (large white x (Landrace x Duroc)) sows and their 132 piglets were housed in conventional farrowing crates (section 2.1) and randomly allocated to one of two treatments.

Treatment M14 - Piglets mixed at 14 days of age.

Treatment M28 – Control piglets remained in litter groups until weaning (28 days)

Two replicates were carried out and each treatment contained three sows and litters. Mixing of piglets was carried out as previously described in section 3.2.1 by the removal of boards between each pen. Standard commercial practices were carried out within 24 hours after birth (section 2.1). Weaning was carried out on day 28 when the piglets were moved from the farrowing crates to standard flat deck accommodation post-weaning (section 2.1). Each treatment was housed in a single pen as an entire group.

4.2.2 Performance

Piglets were weighed after farrowing (day 0), then on days 7, 14 (immediately prior to mixing), 17 (3 days post-mixing), 21, 28 (weaning), 31, 35, 42, 49 and 56 according to the standard operating procedure described in section 2.2.1.

Creep feed (50:50 mix of Ian Hollows Target 1 and Target 275; Table 4.1) was available to all treatments from day 14 onwards supplied in one trough per litter. Consumption was recorded by daily removal and weighing of any uneaten feed. Post-weaning piglets were fed *ad libitum* on a three-phase feeding regime. Piglets were provided with 3kg/pig of Ian Hollows Target 275 followed by 6kg/pig of Ian Hollows Target 4 and then onto Lloyds Grower feed. Feed was weighed back on the piglet weighing days to determine feed consumption and feed conversion ratio. Dietary specifications can be found in Table 2.3 and proximate analysis was also carried out on the diets (Table 4.1) to check actual composition of the diets according to the methods outlined in section 2.3.

Table 4.1 *Proximate analysis of diets provided pre- and post-weaning.*

	Target 1/ Target275 50:50 mix	Target 275	Target 4	Grower
Proximate analysis				
Dry Matter (g/kg)	912	900	903	882
Crude Protein (g/kg DM)	243	244	244	237
Oil (g/kg DM)	98	89	29	32
Ash (g/kg DM)	64	63	62	56
NDF (g/kg DM)	137	131	198	210
DE (MJ/kg DM)†	16.9	16.9	15.0	15.1

† DE = 17.47 + 0.0079CP + 0.0158Oil – 0.0331Ash – 0.0140NDF
(CP, Oil, Ash and NDF in g/kg DM) based on calculation by MAFF (1993)

4.2.3 Lesion scoring

Each piglet was examined for lesions using a method described in section 2.2.2. Briefly, the body of the pig was split up into six different areas of face, ears, shoulders, flank and rump, tail and feet/legs. Each section was then scored for fresh lesions only and scored accordingly (Table 2.1).

4.2.4 Immune responses

Blood samples were collected from 16 randomly selected piglets (eight from each treatment) according to the methods outlined in section 2.2.3 on days 26, 33, 40 and 47. Plasma was stored at -20°C prior to assessment of humoral immune responses and immune responses to dietary soya antigens. Lymphocytes were isolated by density gradient centrifugation (section 2.4.4) to assess cell mediated immunity.

4.2.4.1 Humoral immune response

Piglets were immunised (intra-muscularly in the neck) at weaning (day 28) with 1ml of 1mg/ml (alum precipitated) keyhole limpet haemocyanin (KLH) as prepared in section 2.4.1 to assess humoral immune response of piglets to this novel antigen. Class and sub-class anti-KLH antibody responses were measured using the direct ELISA method described in section 2.4.2. The inter-assay coefficient of variation was calculated by (s.d/meanOD_{405nm}) x 100 and were 5.2%, 6.0%, 3.6% and 1.7% for IgG_1, IgG_2, IgM and IgA respectively. Immune responses to dietary soya antigens were also assessed to check for hypersensitivity responses to soya in the diet using an ELISA method by Miller *et al.* (1984) as outlined in section 2.4.3. and the inter-assay coefficient of variation was 7.9%.

4.2.4.2 Cell mediated immune response

Cell mediated immune response was measured on isolated lymphocytes (section 2.4.4) using a radioisotope lymphocyte blastogenesis test described in section 2.4.5.1.

4.2.5 Statistical Analysis

Statistical analysis was performed using Genstat for Windows Version 4.1. Live weights were analysed using Antedependence Modelling. Analysis of variance was performed on all other performance data. Treatment means were compared using a protected Least Significant Difference (LSD) (Snedecor and Cochran, 1993). Statistical significance was accepted at $P<0.05$. In the farrowing house, the sow and her litter were identified as the experimental unit (d.f. = 11) but in the flat deck the whole pen was used as the experimental unit (d.f. = 1). For immunological data individual piglets were the experimental unit (d.f. = 12).

4.3 RESULTS

4.3.1 Performance

Mortality (4.5%) and morbidity (5%) levels were not significantly different between treatments at any time during the trial period. Repeated measures analysis showed no significant effect of mixing pre-weaning on piglet live weight over time (P=0.430). Antedependence modelling of live weights indicates that an order 1 model was required. There was no significant effect of mixing pre-weaning on live weight either pre-weaning or immediately post-weaning (Table 4.2). However, piglets mixed at 14 days of age were significantly heavier compared with piglets mixed at weaning on day 42 (P=0.006) and also on day 56 (P=0.030).

Table 4.2 *Effect of mixing piglets prior to weaning at 14 days of age on piglet live weight*

	Mixed at 14 days	Mixed at weaning	s.e.d	Significance
Live weight (kg) †				
Birth	1.45	1.54	0.131	NS
Day 7	2.86	2.82	0.140	NS
Day 14	4.76	4.91	0.980	NS
Day 17	5.72	5.80	0.715	NS
Day 21	6.78	6.87	0.102	NS
Day 28 (weaning)	8.75	9.15	0.302	NS
Day 31	9.41	9.30	0.150	NS
Day 35	10.02	10.13	0.193	NS
Day 42	12.78	11.77	0.280	0.006
Day 49	14.99	15.52	0.580	NS
Day 56	19.98	19.28	0.271	0.030

† Means adjusted for the previous weight as a covariate for Antedependence model order 1

There was no significant effect of mixing pre-weaning on piglet performance pre-weaning (Table 4.3). Post-weaning, piglets mixed at 14 days grew significantly faster compared with piglets mixed at weaning from day 35-42 (P=0.005) and from day 49-56 (P=0.003). In the entire post-weaning period there was a tendency for piglets mixed at 14 days to grow faster than piglets mixed at weaning (P=0.065).

Table 4.3 *Effect of mixing on piglet pre- and post-weaning daily live weight gain (DLWG)*

	Mixed at 14 days	Mixed at weaning	s.e.d	Significance
DLWG (g/day)				
Days 0-7†	209	195	30.0	NS
Days 8-14†	273	279	17.1	NS
Days 15-17†	281	320	13.7	NS
Days 18-21†	256	276	25.4	NS
Days 22-28†	274	276	24.8	NS
Days 29-31‡	139	101	29.2	NS
Days 32-35‡	168	192	53.8	NS
Days 36-42‡	393	236	40.4	0.005
Days 43-49‡	394	457	44.6	NS
Days 50-56‡	693	558	33.1	0.003
Days 0-28†	262	266	11.5	NS
Days 29-56‡	409	351	27.1	0.065
Days 0-56†	331	313	12.1	NS

† Means adjusted for birth weight as a covariate ‡ Means adjusted for weaning weight as a covariate

There was no significant effect of mixing at 14 days of age on creep intake or post-weaning feed intake (Table 4.4). Creep intakes were very low prior to weaning therefore the 2 week creep feeding period was combined to assessed the total creep intake. There was also no significant effect on feed conversion ratio throughout the trial. However, from day 43-49 there was a tendency for piglets mixed at 14 days of age to have a higher FCR and therefore not to be converting food as efficiently as piglets mixed at weaning (P=0.088).

Table 4.4 *Effect of mixing pre-weaning on creep intakes, post-weaning feed intakes and feed conversion ratios*

	Mixed at 14 days	Mixed at weaning	s.e.d	Significance
Creep intake (g/pig/day)				
Day 14-28	16	17	3.0	NS
Average feed intake (g/pig/day)				
Day 28-35	186	194	62.7	NS
Day 36-42	447	385	38.2	NS
Day 43-49	629	591	16.0	NS
Day 50-56	923	862	51.8	NS
FCR				
Day 28-35	1.25	1.21	0.506	NS
Day 36-42	1.23	1.45	0.174	NS
Day 43-49	1.72	1.31	0.057	0.088
Day 50-56	1.41	1.48	0.035	NS

4.3.2 Lesion scores

Prior to any mixing, lesion scores were assessed to give a baseline level of lesions as seen on day 7. Increases in lesion scores (Figure 4.1) were observed on day 17 when piglets mixed at 14 days had significantly higher lesion scores compared with piglets that had not yet been mixed or weaned (*P*=0.005).

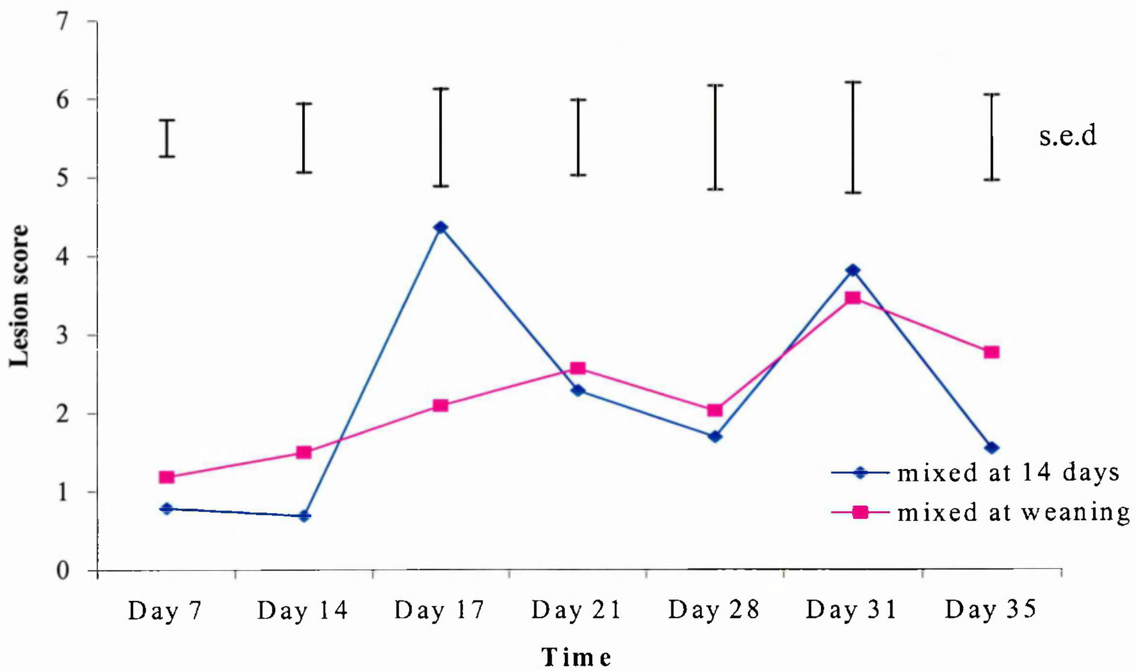

Figure 4.1 Effect of mixing prior to weaning on piglet total body lesion score

There was no significant difference in the increase in lesion scores observed at post-weaning although on day 35 a tendency was observed for piglets mixed at weaning to have a higher lesion scores compared to piglets mixed at 14 days (*P*=0.051).

4.3.3 Immune responses

4.3.3.1 Anti-KLH immune response

Piglets mixed at 14 days of age had a significantly lower anti-KLH IgG_1 antibody response compared with piglets not mixed until weaning on day 40 (*P*<0.001). No other significant effects of mixing were apparent on anti-KLH IgG_1 response post-weaning (Table 4.5 and Figure 4.2).

Table 4.5 *Effect of mixing prior to weaning on piglet Anti-KLH IgG_1 antibody response post-weaning*

	Mixed at 14 days	Mixed at weaning	s.e.d	Significance
IgG_1 Response (OD_{405nm})				
Day 26	0.220	0.207	0.0149	-
Day 33 †	0.217	0.211	0.0120	NS
Day 40 †	0.885	1.536	0.0964	<0.001
Day 47 †	0.863	1.200	0.1519	0.062

† Means adjusted for day 26 as a covariate

There was no significant effect of mixing on anti-KLH IgG_2 antibody response at any time point (Table 4.6 and Figure 4.3).

Table 4.6 *Effect of mixing prior to weaning on piglet Anti-KLH IgG_2 antibody response post-weaning*

	Mixed at 14 days	Mixed at weaning	s.e.d	Significance
IgG_2 Response (OD_{405nm})				
Day 26	0.217	0.266	0.0121	-
Day 33 †	0.268	0.232	0.0289	NS
Day 40 †	0.618	0.828	0.1477	NS
Day 47 †	0.527	0.696	0.1434	NS

† Means adjusted for day 26 as a covariate

Piglets mixed at weaning had a significantly higher anti-KLH IgM response (Table 4.7 and Figure 4.4) compared to piglets mixed prior to weaning at 14 days of age on day 40 (P<0.001).

Table 4.7 *Effect of mixing prior to weaning on piglet Anti-KLH IgM antibody response post-weaning (-log_{10} transformation)*

	Mixed at 14 days	Mixed at weaning	s.e.d	Significance
IgM Response (OD_{405nm}) (untransformed means)				
Day 26	0.678 (0.214)	0.639 (0.231)	0.0262	-
Day 33 †	0.537 (0.295)	0.535 (0.295)	0.0141	NS
Day 40 †	0.224 (0.612)	0.052 (0.887)	0.0259	<0.001
Day 47 †	0.469 (0.373)	0.328 (0.499)	0.0788	NS

† Means adjusted for day 26 as a covariate

Piglets mixed at weaning had significantly higher anti-KLH IgA antibody responses compared with piglets mixed at 14 days of age on days 40 (P=0.008) and 47 (P=0.010) (Table 4.8 and Figure 4.5).

Table 4.8 *Effect of mixing prior to weaning on piglet Anti-KLH IgA antibody response post-weaning*

	Mixed at 14 days	Mixed at weaning	s.e.d	Significance
IgA Response (OD_{405nm})				
Day 26	0.196	0.230	0.0115	-
Day 33 †	0.251	0.262	0.0137	NS
Day 40 †	0.353	0.489	0.0324	0.008
Day 47 †	0.289	0.456	0.0479	0.010

† Means adjusted for day 26 as a covariate

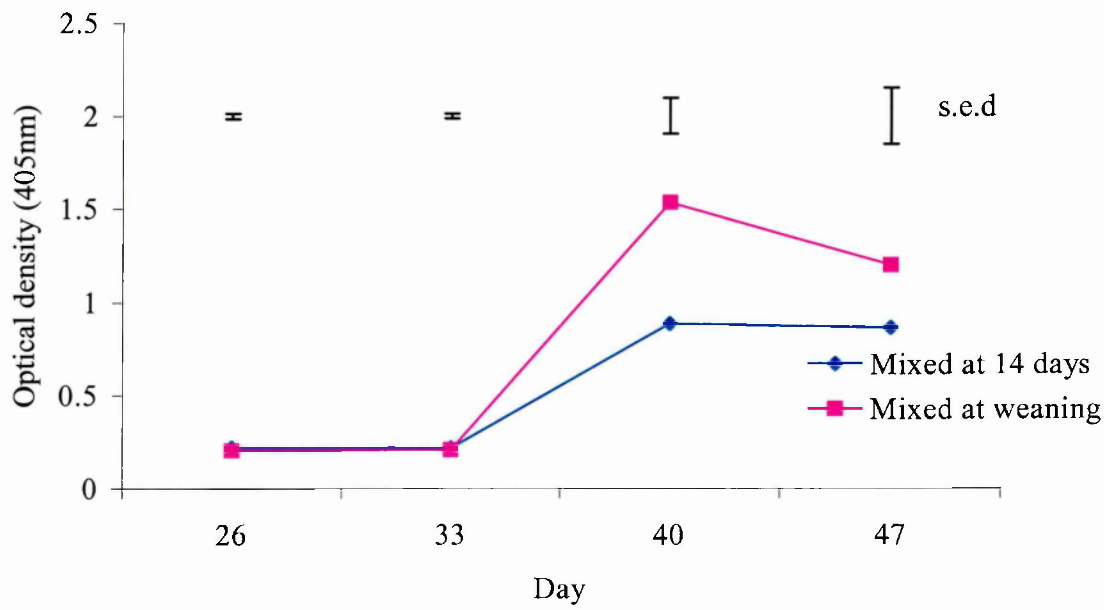

Figure 4.2 Effect of mixing pre-weaning at 14 days of age on piglet anti-KLH IgG_1 response

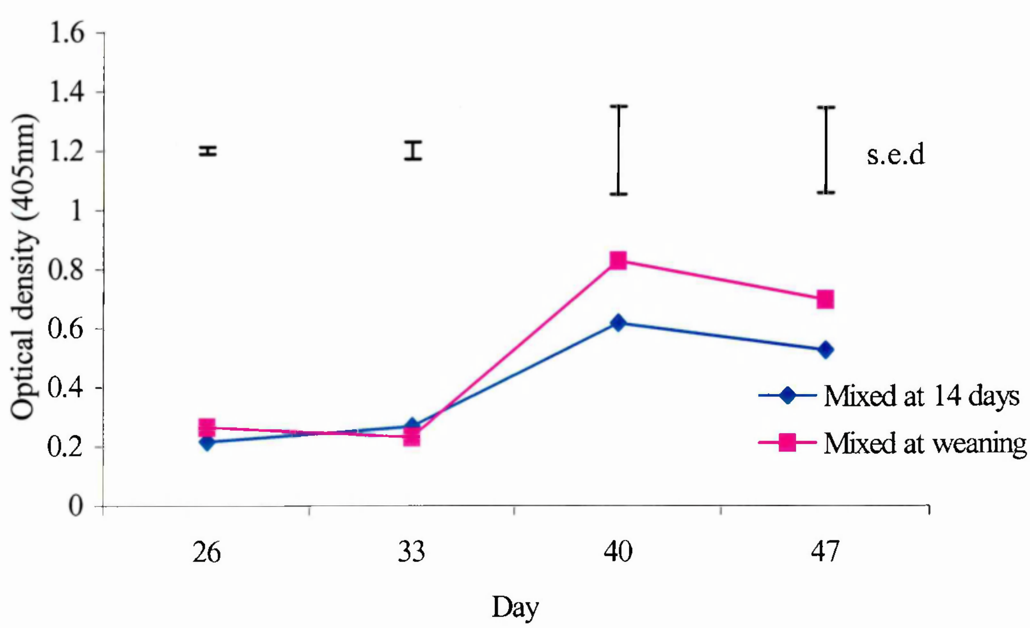

Figure 4.3 Effect of mixing pre-weaning at 14 days of age on piglet anti-KLH IgG_2 response

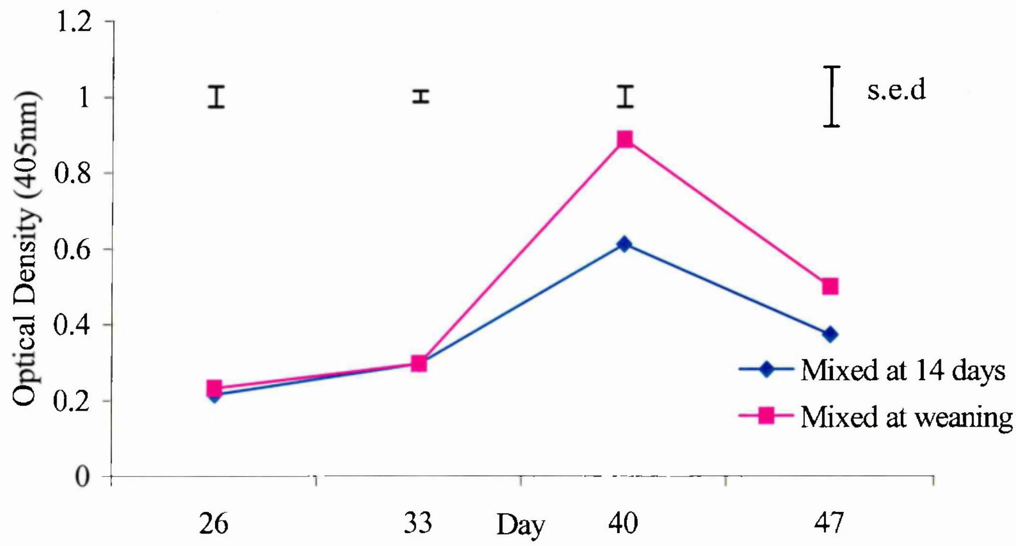

Figure 4.4 Effect of mixing pre-weaning at 14 days of age on piglet anti-KLH IgM response

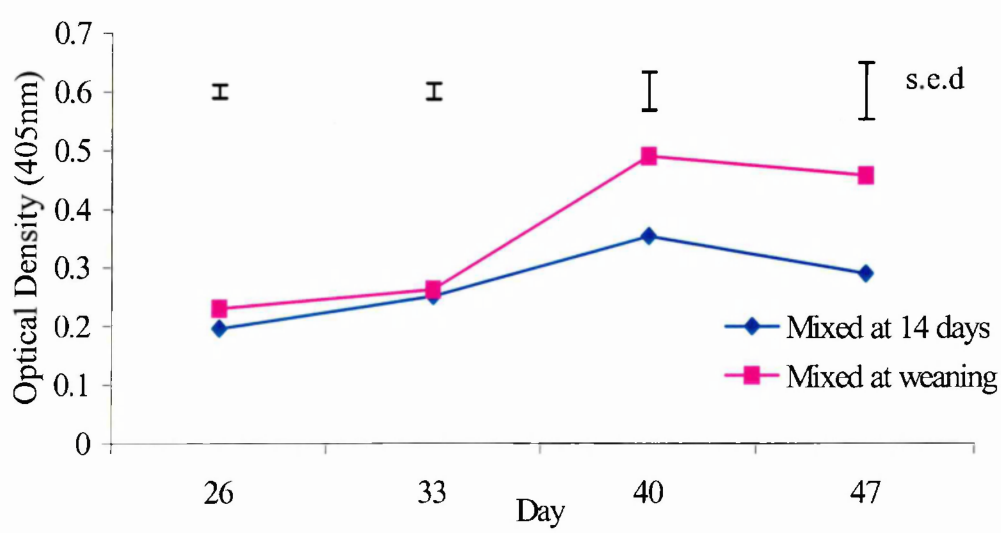

Figure 4.5 Effect of mixing pre-weaning at 14 days of age on piglet anti-KLH IgA response

4.3.3.2 Anti-soya IgG immune response

Piglets mixed pre-weaning at 14 days of age had significantly lower anti-soya IgG antibody responses compared with piglets mixed at weaning (P=0.013) on day 47 (Table 4.9 & Figure 4.6).

Table 4.9 *Effect of mixing prior to weaning on piglet anti-soya IgG immune response to dietary soya antigens*

	Mixed at 14 days	Mixed at weaning	s.e.d	Significance
Anti-soya Response (OD_{405nm})				
Day 26	0.076	0.061	0.0245	-
Day 33 †	0.042	0.083	0.0339	NS
Day 40 †	0.112	0.109	0.0348	NS
Day 47 †	0.097	0.160	0.0212	0.013

† Means adjusted for day 26 as a covariate

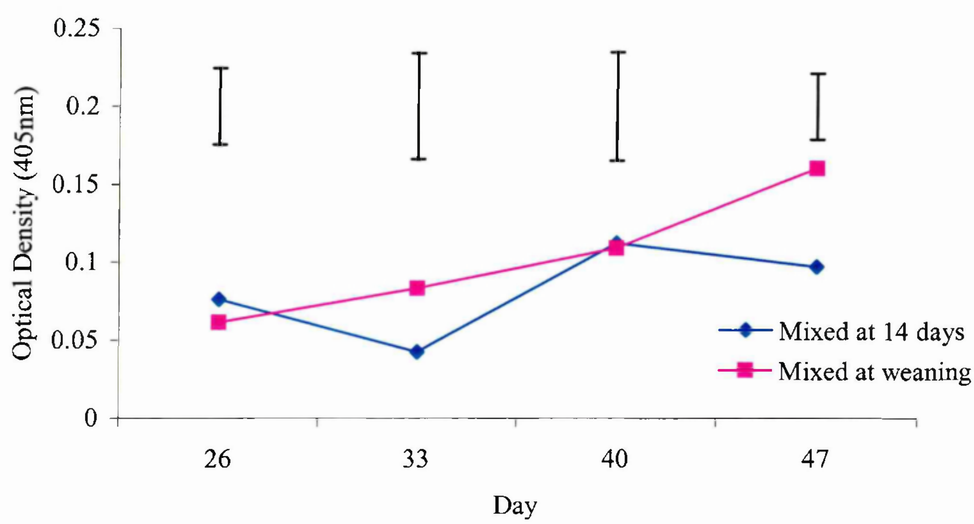

Figure 4.6 Effect of mixing pre-weaning at 14 days of age on piglet anti-soya antibody response.

4.3.3.3 Cell mediated immune response

There was no significant effect of mixing on the unstimulated or stimulated lymphocytes (Table 4.10). However the stimulation index (mean CPM of stimulated cells – mean CPM of unstimulated cells) shows that piglets mixed at 14 days of age had a significantly higher lymphocyte blastogenic response compared with piglets mixed at weaning on day 5 post-weaning (*P*=0.022).

Table 4.10 *Effect of mixing prior to weaning on in vitro lymphocyte blastogenesis test responses to Concanavalin A (Con A) and a control (RPMI 1640)*

	Mixed at 14 days	Mixed at weaning	s.e.d	Significance
Lymphocyte response to control ($\log_e$ mean CPM)				
Day –2	3.11	2.88	0.117	NS
Day 5	3.33	3.38	0.184	NS
Day 19	2.99	3.04	0.055	NS
Lymphocyte response to Con A ($\log_e$ mean CPM)				
Day –2	5.25	4.82	0.537	NS
Day 5	6.73	6.66	0.461	NS
Day 19	5.93	5.95	0.259	NS
Stimulation Index ($\log_e$ (mean CPM ConA - mean CPM Control))				
Day –2	5.05	5.41	0.495	NS
Day 5	6.65	4.61	0.797	0.002
Day 19	5.86	5.18	0.488	NS

4.4 DISCUSSION

This trial was carried out with the emphasis on the effect of mixing pre-weaning on the immune status of the piglet. As the previous chapter discussed the effect of mixing pre-weaning on performance and lesion scores, these areas will only be discussed briefly.

4.4.1 Performance

The positive effect of mixing at 14 days of age appears to be reduced compared with the results from chapter 3 where significant improvements in daily live weight gain were apparent throughout the post-weaning period. This may be due to the small number of piglets used in this trial limiting the performance effects. However, these results do still support the hypothesis that mixing at 14 days of age improves post-weaning performance.

4.4.2 Lesion scores

The lack of a significant reduction in the levels of lesion scores post-weaning was surprising as earlier results showed that mixing at 14 days of age reduced the levels of aggression and therefore skin damage post-weaning. It would appear that the change of environment at weaning affected the piglets' social hierarchy but the reduction in new lesions observed 7 days post-weaning may indicate that less fighting was required for piglets previously mixed to redefine the dominance hierarchy.

4.4.3 Immune responses

A typical primary immune response to a novel antigen was observed in all the piglets' post-immunisation (Bourne, 1976) yet in all four antibody sub-classes piglets mixed at weaning had higher antibody responses than piglets mixed at 14 days of age. This supports previous studies using KLH/alum reported by Pollock *et al.* (1992), Mackenzie (1994) and Mackenzie *et al.* (1997) where a significant increase in antibody responses were observed associated with the stress of weaning in calves. This corresponds with these results which show that piglets mixed at 14 days of age would have had a stressor removed from the point of weaning and therefore should be less stressed at weaning compared to the piglets mixed at weaning who also had an increase in antibody response.

It has been reported that stress caused by weaning can alter the way the immune system functions so that production of IgG and IgA is favoured over IgM (Rook *et al.*, 1994), hence weaning stressors may cause reduced levels of IgM compared to IgG and IgA. It is also known that IgG is the dominant antibody class in response to alum-precipitated antigens such as KLH (Roitt *et al.*, 1992) and it is clear that IgG_1 and IgG_2 had the highest response. IgA continues to remain at low absorbance levels although there is a difference between the mixed and unmixed groups this low level maybe due to IgA being more specific to mucosal immune function. Kelly *et al.* (2000) found similar levels of IgG_1 two weeks post-weaning but lower levels of IgG_2, IgM and IgA. This may have been because piglets were weaned at 3-4 weeks of age and weighing 6.4kg live weight while piglets weaned in this study were approximately 9kg at 4 weeks of age.

Blecha *et al.* (1983) reported that weaning at 5 weeks of age had no significant effect on cell-mediated immunity. However, piglets weaned at 2 and 3 weeks of age had reduced lymphocyte blastogenic responses and piglets weaned at 4 weeks had a reduced 24 hour skin test response which was 14 % lower than the unweaned controls therefore indicating that there may be an effect of weaning on cell-mediated immunity.

The reduced stimulation index for piglets mixed at weaning compared with piglets mixed pre-weaning, on day 5 post-weaning suggests that mixing pre-weaning may allow the piglet's cell-mediated immunity to cope with the other stressors involved with weaning. This increase in cell-mediated immunity in piglets mixed at 14 days of age and the reduction in their humoral antibody response compared with piglets mixed at weaning supports the hypothesis of a converse relationship between cell-mediated immunity and humoral immune responses to a single stressor (Hessing *et al.*, 1995).

4.5 CONCLUSION

The effect of mixing at weaning on the immune system of piglets post-weaning appears to cause an increase in the number of antibodies produced in response to a novel antigen. This supports the hypothesis that by removing a stressor away from the point of weaning can reduce the level of stress and that mixing piglets at 14 days of age may promote improved performance, welfare and immune status post-weaning. There is also evidence that a single stressor can cause a converse relationship between cell-mediated and humoral immune responses.

CHAPTER 5. THE EFFECT OF MIXING PRE-WEANING AND AGAIN AT WEANING ON PIGLET PERFORMANCE

5.1 INTRODUCTION

The mixing of non-litter mates is usually standard practice in commercial enterprises yet it is one of the largest stressors observed at weaning and leads to increased aggression between unfamiliar individuals to determine the dominance hierarchy of the newly formed group (Friend *et al.*, 1983).

One method that may be used to minimise the stress of weaning other than to maintain litters at weaning is to mix piglets pre-weaning such that it conditions them to mixing again at weaning and in later stages (Pluske and Williams, 1996b; Wattanakul *et al.*, 1997b). As seen in chapter 3, grouping piglets prior to weaning can reduce aggression post-weaning and it has also been found to increase the number of individuals that can be recognised therefore decreasing the number of unfamiliar pigs within the newly formed group at weaning (Stookey and Gonyou, 1998).

There are numerous hypotheses relating to the effect of positive and negative stressors on performance parameters such as mixing at weaning and North and Stewart (2000) showed a tendency for the piglet's food intake to be stimulated by mixing prior to weaning and again at weaning. From chapter 3 it was identified that mixing at 14 days of age was the optimum time to mix pre-weaning without detrimental affects on pre-weaning growth and behaviour combined with improved post-weaning performance. Therefore the aim of this trial was to study the effects of mixing piglets pre-weaning and/or at weaning and of creep feed availability on piglet performance and skin lesions.

5.2 MATERIAL AND METHODS

5.2.1 Animals and Housing

Twenty-four PIC Camborough 15 (Large White x (Landrace x Duroc)) sows and their 109 piglets were housed in conventional farrowing crates (Section 2.1) and randomly allocated in a 3 x 2 factorial design with the following treatments:

M14/28 – Mixed 14 days prior to weaning (≈ 14 days of age) and mixed again at weaning (28 days of age)

M14 – Mixed 14 days prior to weaning (≈ 14 days of age) but not at weaning

M28 – Not mixed prior to weaning but mixed at 28 days of age (weaning)

Half of the treatment groups had access to a commercial creep diet (C) from 21 days of age and the other half had no access to creep (NC) prior to weaning.

Mixing of piglets was carried out by the removal of the boards between two pens so that the piglets from two litters could mix together whilst the sows remained confined in the farrowing crate. Standard commercial procedures were carried out approximately 24 hours after birth as described in section 2.1. Weaning took place at 28 days when the piglets were removed from the farrowing pen into fully slatted flat deck accommodation (Section 2.1). In the flat decks the piglets were housed in pens as treatment groups (i.e. two litters).

5.2.2 Performance

Piglets were weighed after farrowing (day 0), then on days 7, 14 (before mixing), 17 (3 days post-mixing), 21, 28 (day of weaning), and on days 31, 35, 42, 49 and 56 according to the standard operating procedure (section 2.2.1). Creep feed (50:50 Mix of Ian Hollows Target 1:Target 275; Table 5.1) was weighed and supplied to the appropriate treatment (C) in one trough per litter in each pen from day 21 to weaning. Post-weaning piglets were fed *ad libitum* on a three stage feeding regime of commercial weaner diets (Ian Hollows Diets and Lloyds grower diet; Table 5.1). Piglets were supplied with 3kg/pig of Target 275 followed by 6kg/pig of Target 4, then onto the Grower diet. Food was weighed weekly post-weaning.

Table 5.1 *Proximate analysis of diets provided pre- and post-weaning.*

	Target 1/ Target 275 50:50 mix	Target 275	Target 4	Grower
Proximate analysis				
Dry Matter (g/kg)	912	897	901	876
Crude Protein (g/kg DM)	239	242	236	220
Oil (g/kg DM)	104	83	19	22
Ash (g/kg DM)	68	69	64	52
NDF (g/kg DM)	118	145	209	163
DE (MJ/kg DM)†	17.1	16.4	14.6	15.6

† DE = 17.47 + 0.0079CP + 0.0158Oil – 0.0331Ash – 0.0140NDF
(CP, Oil, Ash and NDF in g/kg DM) based on calculation by MAFF (1993)

5.2.3 Lesion Scoring

Lesion scores were assessed at each weighing up to day 35 using the method described in section 2.2.2. Lesion scores were totalled up to give a total body score for analysis and only fresh lesions were recorded using the classifications shown in Table 2.1.

5.2.4 Statistical analysis

Statistical analysis was performed using Genstat for windows (Version 4.1). Liveweight data were analysed using antedependence modelling and all other performance data analysed by analysis of variance. The protected least significant difference (LSD) was calculated to compare treatment means (Snedecor and Cochran, 1993) and statistical significance was accepted at $P<0.05$. The sow and her litter were identified as the experimental unit prior to weaning (d.f. = 18) and the pen as a whole was used as the experimental unit post-weaning (d.f. = 5).

5.3 RESULTS

5.3.1 Performance

Antedependence modelling of liveweight data identified that an order 1 model was appropriate and therefore only the previous weight was used as a covariate (Table 5.2). There was no significant mixing x creep interaction at any point throughout the experimental period. A significant difference was observed at weaning when comparing the main effect of mixing with piglets mixed at 14 days of age being significantly heavier than piglets kept as intact litters pre-weaning (8.95kg vs.9.13kg vs. 8.84kg, for M14/28, M14 and M28 respectively, s.e.d 0.101, *P*=0.020).

Table 5.2 *Effect of mixing piglets pre-weaning and/or at weaning and creep availability on live weight*

	Treatment							Significance		
	M14/ 28 C	M14/ 28 NC	M14 C	M14 NC	M28 C	M28 NC	s.e.d	mix	creep	mix x creep
Live weight (kg)[†]										
Birth	1.73	1.61	1.71	1.40	1.47	1.69	0.144	-	-	-
7	3.36	2.60	3.10	2.56	3.11	2.98	0.241	-	-	-
14	4.94	4.87	4.97	4.79	5.04	4.95	0.116	NS	-	-
17	6.17	5.77	6.09	5.72	5.84	5.95	0.106	NS	-	-
21	7.15	7.05	7.21	7.26	7.05	7.19	0.169	NS	NS	NS
28	9.10	8.80	9.20	9.05	8.75	8.93	0.166	0.020	NS	NS
31	9.09	9.37	9.21	9.54	9.09	9.37	0.298	NS	NS	NS
35	10.35	10.17	10.49	10.52	10.27	10.39	0.301	NS	NS	NS
42	12.74	12.72	12.90	12.41	12.78	12.72	0.497	NS	NS	NS
49	15.85	15.84	16.13	16.03	15.71	16.04	0.525	NS	NS	NS
56	20.05	20.05	20.21	20.32	19.16	20.20	0.609	NS	NS	NS

[†] Means adjusted for the previous weight as covariates for Antedependence model order 1

There was no significant mixing x creep interaction on daily live weight gain (Table 5.3). Piglets mixed at 14 days of age had a significantly higher daily live weight gain compared with piglets mixed at weaning from day 22 to weaning (277g/day vs. 306 vs. 261, for M14/28, M14 and M28 respectively, s.e.d 15.6, *P*= 0.025). From day 50 onwards piglets mixed at 14 days had a significantly higher daily live weight gain than piglets mixed at weaning (582g/day vs. 623 vs. 538, for M14/28, M14 and M28 respectively, s.e.d 28.6, P=0.026). Creep availability also had a significant effect of daily live weight gain post-weaning from 49 days onwards with piglets that had no access to creep growing faster than the piglets that had access to creep (532g/day vs. 630, for creep and no creep respectively, s.e.d 33.9, P= 0.001).

Table 5.3 *Effect of mixing piglets pre-weaning and/or at weaning and creep availability on daily live weight gain (DLWG)*

	Treatment							P-value		
	M14/ 28 C	M14/ 28 NC	M14 C	M14 NC	M28 C	M28 NC	s.e.d	Mix	Creep	Mix x creep
Daily live weight gain (g/day)										
0-7	201	165	197	160	206	199	17.1	-	-	-
8-14	290	268	288	240	280	275	22.2	-	-	-
15-17	342	281	330	286	305	332	18.9	NS	-	-
18-21	306	279	320	305	284	314	22.0	NS	-	-
22-28	297	258	313	300	252	271	22.8	0.025	NS	NS
29-31	-25	143	28	112	61	70	78.3	NS	NS	NS
32-35	223	259	247	348	266	267	53.4	NS	NS	NS
36-42	368	296	389	270	317	344	48.7	NS	NS	NS
43-49	408	449	494	486	422	479	79.0	NS	NS	NS
50-56	543	621	581	664	471	604	49.4	0.026	0.001	NS
0-28	279	251	283	260	261	269	12.1	NS	0.061	NS
29-56	366	393	405	416	352	403	46.4	NS	NS	NS
0-56	333	315	355	338	303	340	19.5	NS	NS	NS

There were no significant interactions between mixing and creep availability at any age on creep intake, feed intake or feed conversion ratio (Table 5.4). By day 49 onwards piglets mixed prior to weaning and mixed twice (at 14 days of age and at weaning) had significantly higher feed intakes than piglets mixed at weaning (842 vs. 814 vs. 722 (g/pig/day), for M14/28, M14 and M28 respectively, s.e.d 27.2, P=0.016). There were no significant effects of mixing or creep availability on feed conversion ratio.

Table 5.4 *Effect of mixing piglets pre-weaning and/or at weaning and creep availability on feed intakes and feed conversion ratios*

	Treatment							*P*-value		
	M2X C	M2X NC	M14 C	M14 NC	MW C	MW NC	s.e.d	Mix	Creep	Mix x creep
Average feed intake (g/pig/day)										
w-35	208	225	203	262	214	221	27.0	NS	NS	NS
35-42	395	444	402	359	403	416	24.0	NS	NS	NS
42-49	606	646	659	595	679	621	50.7	NS	NS	NS
49-56	812	872	835	793	696	747	38.5	0.016	NS	NS
FCR										
w-35	1.22	1.39	1.14	1.30	1.41	1.14	0.272	NS	NS	NS
35-42	1.24	1.38	1.08	1.29	1.28	1.26	0.213	NS	NS	NS
42-49	1.38	1.65	1.29	1.36	1.70	1.29	0.294	NS	NS	NS
49-56	1.32	1.69	1.36	1.30	1.56	1.26	0.173	NS	NS	NS

5.3.2 Lesion scores

There was no significant mixing x creep interaction on total body lesion scores at any stage during the experimental period (Figure 5.1). There were also no significant effects of mixing or creep availability on total body lesions scores.

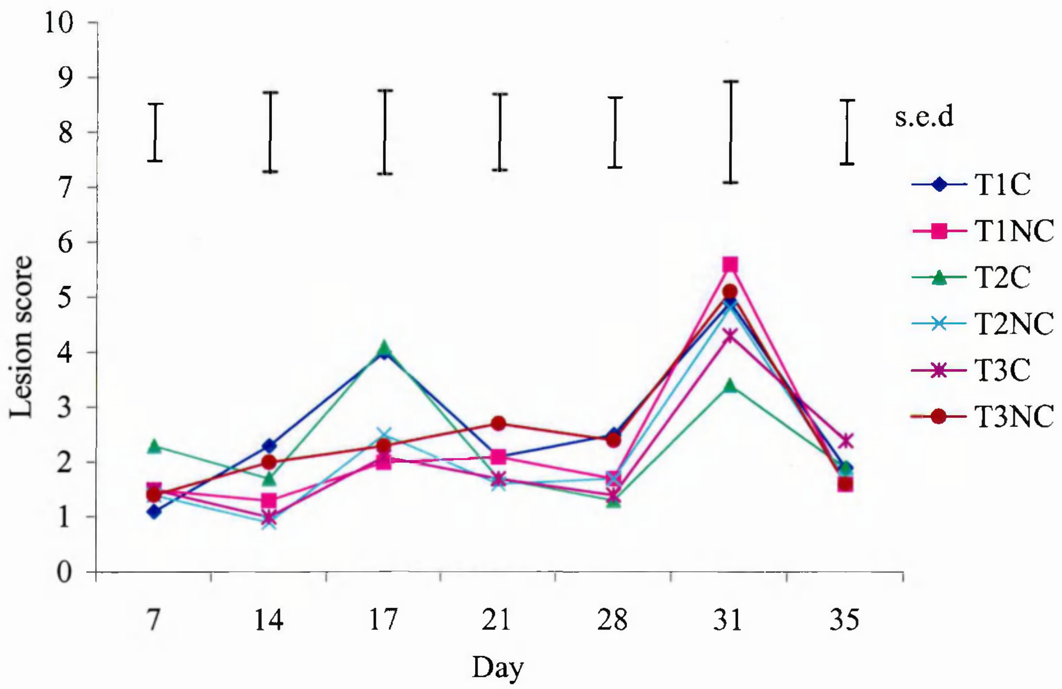

Figure 5.1 Effect of mixing pre-weaning and/or at weaning and creep availability on total body lesion score of piglets.

5.4 DISCUSSION

5.4.1 Performance

The observation that piglets mixed pre-weaning had similar daily live weights gains as piglets conventionally weaned suggests that mixing prior to weaning does not cause disruption to suckling patterns due to cross-suckling which has been previously reported by Wattanakul *et al.* (1996). In the post-weaning period a growth check was still observed in all groups, indicating that mixing pre-weaning is not the only stressor involved in the weaning process.

Similar to the effects observed in chapter 3, improvements in long term post-weaning performance were seen when mixing at 14 days of age compared to mixing piglets at weaning. However, piglets that were mixed twice did not show the improvement in daily live weight gain observed by North and Stewart (2000) which reported improvements in performance when mixing piglets twice although piglets were mixed at 11 days of age instead of 14 days of age as in this experiment.

When mixing at 11 days of age and allowing three litters to mix instead of two, a disruption to suckling and a drop in live weight pre-weaning was reported as sows were disturbed more often by fighting and foreign piglets at the udder during suckling (Wattanakul *et al.*, 1997b) yet piglets mixed pre-weaning were significantly heavier at weaning compared with piglets left as individual litters. A reduction in the nursing frequency by the sows over the four weeks (Pedersen *et al.*, 1998) may explain the reduction in daily live weight gain observed across all treatments in the last week pre-weaning.

Creep intake in the pre-weaned piglet has been reported to be highly variable both between and within litters (Pajor *et al.*, 1991; Fraser *et al.*, 1994; Delumeau and Meunier -Salaun, 1995; Kavanagh *et al.*, 1996) which may have a knock on effect post-weaning as the gut may become sensitised by a small amount of creep (Hampson and Fu, 1988). The change in diet post-weaning may cause poorer growth due to the increased sensitivity and the effect of creep on the development of the gut (Barnett *et al.*, 1989). Another possible reason is that the novelty of the diet may have worn off by weaning for the piglets with previous access and therefore, the piglets without access tend to investigate the feed more post-weaning and social facilitation encourages other piglets to investigate and eat as well (Keeling and Hurnik, 1996).

North and Stewart (2000) reported that mixing pre-weaning and again at weaning appeared to increase the piglets feed intake post-weaning and this is also apparent where piglets mixed prior to weaning consumed more feed compared with piglets mixed at weaning regardless of piglets being mixed once or twice. Wattanakul *et al.* (1997b) reported that piglets mixed pre-weaning at 11 days of age provided similar results of improved feed intakes in the second week post-weaning when compared with piglets mixed at weaning yet Pluske and Williams (1996b) reported that mixing three litters at 10 days of age had no such effect on post-weaning feed intakes. No such effect was observed during this experiment and the limited results, whilst not indicating a detrimental effect of mixing prior to weaning on feed intake, do not support the positive increases in feed consumption previously reported.

5.4.2 Lesion scores

There was no significant effect of mixing at any time on lesion scores. This is in contrast to the results reported in chapter 3 where a significant relationship between mixing and lesion scores was noted and suggested that mixing pre-weaning reduced post-weaning lesion scores, and thereby indicating a reduction in aggression during the immediate post-weaning period. However, this may be the result of mixing of only two litters as opposed to three and so reducing the number of individuals fighting to establish the new dominance hierarchy.

Gonyou (1997) identified that litters and previously mixed piglets can continue to recognize each other in the period immediately after weaning. Therefore fights should be brief and at a minimum. This generally explains the reduction in lesion scores previously seen as most damaging behaviour such as bites are known to occur later in a fight (McGlone, 1985). It is not known why this was not apparent for piglets mixed pre-weaning. Allowing litters to mix prior to weaning reduces the aggressiveness observed post-weaning. This is beneficial to the welfare of the piglets because younger piglets (1-2 weeks of age) cause less damage to each other than older piglets (3-5 weeks of age) (Pitts *et al.*, 2000).

5.5 CONCLUSIONS

These results are consistent with previous studies showing that mixing different litters prior to weaning is a simple management procedure that can significantly improve long term post-weaning performance. Also access to creep in the last week prior to weaning can be detrimental to post-weaning performance as variable uptake of creep by individual piglets causes depressed growth post-weaning. Further work needs to be carried out to identify possible creep management techniques to improve creep intake.

CHAPTER 6. THE EFFECT OF RELOCATING PIGLETS AT DIFFERENT AGES POST-WEANING AND MIXING PRE-WEANING ON PIGLET PERFORMANCE AND IMMUNE FUNCTION

6.1 INTRODUCTION

The post-weaning environment has a large influence on the expression of abnormal behaviours in the newly weaned piglet (Bøe, 1993). It has been observed that the barren environment to which newly weaned pigs are moved increases the frequency and intensity of unnatural behaviours and also increases the risk of health problems (Meunier-Salaün and Dantzer, 1990).

Pigs are moved to new environments several times during their life and often each time they are moved, they are grouped with unfamiliar pigs causing a new social hierarchy to be formed (Varley, 1995). It is thought that the enrichment of each new environment, e.g. using novel items, decreases the aggression between individuals and enables relocation to be a positive management tool not a stressor (Schaefer *et al.*, 1990). The introduction of playthings such as tyres or chains is being used more frequently on a commercial basis in an attempt to minimise the stress of relocation (Hill *et al.*, 1998). However, familiarity with the inanimate objects appears to reduce the effectiveness of the object to enrich the environment (Schaefer *et al.*, 1990).

Relocation at weaning is the first change of environment for the commercially reared piglet. Housing systems used at weaning can vary greatly with floor type, the use of straw and hiding places all of which have been reported to affect the behaviour and performance of the pigs (Petersen *et al.*, 1995; Amory, 2001). McKinnon *et al.*

(1989) concluded that pigs housed on solid flooring that are provided with small quantities of straw compared to fully perforated or part-solid floors had improved welfare and health (e.g. less aggression therefore less open wounds). Bøe (1993) identified that in fully slatted flat deck accommodation which may be considered a barren environment, exploratory behaviour by the piglets was still observed but redirected towards other penmates rather than the environment. This altered exploratory behaviour is thought to indicate poor welfare which may lead to a reduction in performance although Bøe (1993) did not measure any performance parameters.

Puppe *et al.* (1997) examined the effect of familiarity of environment at weaning on agonistic behaviour of piglets weaned at 42 days of age where the piglets remained in the farrowing house for an unspecified time compared with conventionally weaned piglets relocated immediately post-weaning. It was concluded that the newly weaned pig appeared to have more trouble adapting to a new environment than coping with unfamiliar pigs and therefore more research in this area would be beneficial to identify how to minimise the stress of relocation at weaning. Limited work has been carried out on the length of time after weaning that relocation should occur.

As previously reported in chapters 3 and 4 mixing piglets pre-weaning at 14 days of age improved post-weaning performance. The potential of this management technique of mixing pre-weaning may be used in combination with other techniques to minimise the growth check at weaning and will be further assessed in the following chapters. The effect of the first relocation, usually at weaning, during a piglet's life is particularly important as it has the most impact due to the timing of other changes such as loss of the sow and a dietary change. Therefore any attempt to reduce this impact may make a significant improvement in the piglet's ability to cope. It should also be noted that if mixing at 14 days of age there is a subtle change of environment as the piglet have access to more farrowing pens. The aim of this trial was to investigate both the effect of relocating piglets to flat deck accommodation 9 days post-weaning and the combined effect of relocation and mixing pre-weaning at 14 days on performance, behaviour, humoral immunity and gut morphology of the weaned piglet.

6.2 MATERIAL AND METHODS

6.2.1. Animals and Housing

Forty-eight Camborough 15 sows (Large White x (Landrace x Duroc)) and their 461 piglets kept in conventional farrowing crates (section 2.1) were randomly allocated to a 2x2 factorial design (Figure 6.1). Each replicate had three litters per treatment therefore using 12 sows and their piglets.

2x2 FACTORIAL DESIGN

Mixed at 14 days (M14) | Mixed at weaning (M24)

Relocate at Weaning (R24) | Relocate 9 days post-weaning (R33) | Relocate at weaning (R24) | Relocate 9 days post-weaning (R33)

Figure 6.1 Experimental design for relocating post-weaning and mixing pre-weaning

Mixing of piglets was carried out by the removal of the boards as previously described in section 3.2.1 Standard commercial procedures were carried out approximately 24 hours after birth as described in section 2.1.

Weaning took place at 24 days of age when the sows were removed from the farrowing crates. The piglets were either moved to the unfamiliar environment of fully slatted flat deck accommodation (section 2.1) at 24 days of age or remained in the familiar farrowing crates according to the experimental design for a further 9 days until 33 days of age. The piglets remained in their specific treatment groups and were housed as a single group in the flat deck accommodation. The temperature ranges of the farrowing room and flat decks were 19.8-30.1 with an average of 22.5 and 22.8-30.6 with an average of 26.8 respectively.

6.2.2 Performance

Piglets were weighed after farrowing (day 0), then on days 7, 14 (day of mixing), 17, 24 (day of weaning), 27 (3 days post-weaning), 33 (day of relocation), 36 (3 days post-relocation) 40 and 47 according to the standard operating procedure (section 2.2.1).

Creep feed (Target 2, Ian Hollows Diets) was weighed and supplied in one trough per litter in each pen from day 17 onwards. Post-weaning piglets were offered a three-stage feeding regime (Ian Hollows Diets) *ad libitum*. Table 6.1 shows the proximate analysis of the feeds. Each piglet was allocated 2kg of Target 2, 3kg of Target 3 and 4kg of Target 4 and once the treatment allocation had been eaten the next diet was supplied by mixing the new diet with the remains from the previous diet to allow for an adjustment period. Food was weighed back on the weighing days post-weaning and amount eaten determined each day.

Table 6.1 *Proximate analysis of the three diets used in the feeding regime*

	Target 2	Target 3	Target 4
Proximate Analysis			
Dry Matter (g/kg)	886	886	894
Crude Protein (g/kg DM)	223	233	225
Oil (g/kg DM)	54	59	39
Ash (g/kg DM)	72	67	59
NDF (g/kg DM)	157	153	181
DE †	15.5	15.9	15.4

† DE = 17.47 + 0.0079CP + 0.0158Oil – 0.0331Ash – 0.0140NDF
(CP, Oil, Ash and NDF in g/kg DM) based on calculation by MAFF (1993)

6.2.3 Lesion Scoring

Each piglet was examined for lesions at each weighing according to the method described in section 2.2.2. Each section was scored for fresh lesions only and classified accordingly (Table 2.1)

6.2.4 Behavioural Observations

Teat order was assessed before mixing occurred and again on days 1 and 7 post-mixing according to the method outlined in section 2.2.4.3. Cross-suckling of the sows by the piglets was recorded during the teat order observation period post-mixing.

6.2.5 Humoral immune response

Ninety-six piglets (24 per treatment) were immunised (intra-muscularly in the neck) at weaning (24 days of age) with 1ml of 1mg/ml (alum precipitated) keyhole limpet haemocyanin (KLH) (section 2.4.1) to assess the humoral immune response of piglets to a novel antigen. Blood samples were collected according to section 2.2.3 on day 24 (weaning) and again on days 40 and 47. Class and sub-class anti-KLH antibody responses were measured using an ELISA based on the method by Pollock *et al.* (1991) as outlined in section 2.4.2. Inter-assay coefficent of variations were calculated as in section 4.2.4.1 and were 18.2%, 24.0%, 9.4% and 22.1% for IgG_1, IgG_2, IgM and IgA respectively.

6.2.6 Gut Morphology

Twelve piglets (3 per treatment) were slaughtered using a schedule 1 method on the day of relocation and three sections of the small intestine dissected (section 2.5) and stored in 3% Gluteraldehyde for later analysis of crypt dept, villus height and the crypt to villus ratio (section 2.5.1).

6.2.7 Adrenal glands

The adrenal glands were removed after slaughter and any excess fat removed before weighing (section 2.5) to assess any differences in long-term cortisol production as previously observed by Hessing *et al.* (1994) and Beattie *et al.* (2000) who used adrenal weight as a reflection of long-terms levels of cortisol production.

6.2.8 Statistical analysis

Statistical analysis was performed using Genstat for Windows Version 4.1. Live weights were analysed using repeated measures analysis of variance and Antedependence Modelling. Analysis of variance was performed on all other performance data. Treatment means were compared by calculating the Protected Least Significant Difference (LSD) (Snedecor and Cochran, 1993). Statistical significance was accepted at $P<0.05$. In the farrowing house, the sow and her litter were identified as the experimental unit (d.f. = 41) but in the flat deck the whole pen (i.e. treatment) was used as the experimental unit (d.f. = 9).

6.3 RESULTS

6.3.1 Performance

There was no significant difference in the number of piglets per treatment at any time during the trial. There was also no significant effect on piglet mortality (4%) and morbidity (9%) across the treatments.

Repeated measures analysis on live weights showed that there was a significant time x relocation interaction (P=0.041) but no other significant effects were observed. Further analysis of live weights using antedependence modelling determined that an order 2 model needed to be used indicating that the 2 previous live weights should be used as covariates for analysis of variance (Table 6.2).

Table 6.2 *Effect of mixing and relocation on piglet live weights*

	Treatment					Significance		
	M24 R24	M24 R33	M14 R24	M14 R33	s.e.d	mix	relocate	mix x relocate
Live weight (kg)†								
Birth	1.70	1.58	1.55	1.62	0.095	NS	NS	NS
Day 7	3.10	2.89	3.08	3.06	0.154	NS	NS	NS
Day 14	4.96	5.03	5.07	5.10	0.135	NS	NS	NS
Day 17	5.90	5.90	5.90	5.90	0.040	NS	NS	NS
Day 24	7.84	7.97	8.01	7.90	0.142	NS	NS	NS
Day 27	8.19	8.07	8.31	8.01	0.100	NS	0.005	NS
Day 33	9.00	8.78	9.01	8.84	0.125	NS	0.038	NS
Day 36	9.93	9.75	9.86	9.87	0.081	NS	NS	NS
Day 40	11.42	11.49	11.33	11.19	0.135	0.049	NS	NS
Day 47	14.37	14.31	14.51	14.30	0.235	NS	NS	NS

† Means adjusted for previous two weights as covariates according to antedependence modelling order 2

There was no significant mixing x relocation interaction on live weight throughout the trial. However on day 36 there was a trend for piglets mixed and relocated at weaning to be heavier (*P*=0.082) compared with all the other treatments. Piglets mixed at weaning were significantly heavier on day 40 compared with piglets mixed at 14 days of age (11.45kg vs. 11.26, s.e.d 0.092, P=0.049). Piglets relocated to the flat decks at weaning were significantly heavier compared with piglets left in the farrowing room post-weaning on days 27 and 33 (8.23kg vs. 8.04, s.e.d 0.070, P=0.005 and 9.00 vs. 8.81, s.e.d 0.094, P=0.038 respectively).

There was no significant effect of treatment on pre-weaning daily live weight gain (Table 6.3). However, from day 28 to 36 the piglets which remained in the farrowing rooms post-weaning had significantly lower daily live weight gains compared with piglets relocated to the flat deck accommodation (111g/day vs. 138, s.e.d 18.8, P=0.041). It was also observed that piglets relocated at weaning grew significantly faster compared to piglets left in the farrowing rooms over the entire three week post-weaning period (290g/day vs. 262, s.e.d 10.6, P = 0.011).

Table 6.3 *The effect of mixing and relocation on pre- and post-weaning daily live weight gain (DLWG) of piglets*

	Treatment					Significance		
	M24 R24	M24 R33	M14 R24	M14 R33	s.e.d	mix	relocate	mix x relocate
DLWG (g/d)								
0-7†	213	193	210	209	15.6	NS	NS	NS
8-14†	277	270	290	292	20.6	NS	NS	NS
15-17†	277	285	293	285	16.9	NS	NS	NS
18-24†	276	273	303	279	20.7	NS	NS	NS
25-27‡	53	50	91	27	34.4	NS	NS	NS
28-33‡	144	104	133	118	18.2	NS	0.041	NS
34-36‡	338	264	326	315	27.4	NS	0.034	NS
37-40‡	366	413	363	332	31.0	NS	NS	NS
41-47‡	441	412	451	407	33.1	NS	NS	NS
0-24†	261	258	272	269	14.9	NS	NS	NS
25-47‡	290	265	290	259	15.0	NS	0.011	NS
0-47†	278	259	288	266	14.5	NS	NS	NS

† Means adjusted for birth weight as a covariate ‡ Means adjusted for weaning weight as a covariate

Creep feed intakes were extremely low prior to weaning and there was no significant interactive or main effect of treatment on average creep intakes (Table 6.4). However, there was a tendency for piglets mixed prior to weaning to eat more compared with piglets left in intact litters until weaning (22g/day vs. 17, s.e.d 2.7, P=0.096). No significant treatment interactions were observed on average feed intakes or feed conversion ratios (Table 6.4). There were no significant effects of treatment on feed conversion but piglets that remained in the farrowing room ate significantly less from day 28 to 33 compared with piglets relocated at weaning (170g/day vs. 242, s.e.d 22.0, P=0.010). However, once these piglets were relocated to flat deck accommodation on day 33 their feed intakes increased to similar levels of those piglets previously relocated at weaning.

Table 6.4 *Effect of mixing and relocation on creep intake, post-weaning feed intakes and feed conversion ratios*

	Treatment					Significance		
	M24 R24	M24 R33	M14 R24	M14 R33	s.e.d	mix	relocate	mix x relocate
Average creep intakes (g/pig/day)								
17-24	17	17	22	22	3.8	NS	NS	NS
Average feed intakes (g/pig/day)								
24-27	124	105	144	97	27.8	NS	NS	NS
28-33	252	164	232	177	31.1	NS	0.010	NS
34-36	348	306	344	309	47.6	NS	NS	NS
37-40	457	478	392	502	83.3	NS	NS	NS
41-47	599	578	659	601	43.4	NS	NS	NS
FCR								
24-27	0.7	1.2	0.7	1.3	0.83	NS	NS	NS
28-33	1.8	3.5	1.7	2.0	1.31	NS	NS	NS
34-36	1.1	1.2	1.1	1.1	0.10	NS	NS	NS
37-40	1.3	1.2	1.1	1.5	0.20	NS	NS	NS
41-47	1.4	1.3	1.4	1.4	0.13	NS	NS	NS

6.3.2 Lesion scores

There was no significant mixing x relocation interaction on total body lesion scores at any stage during the trial (Figure 6.2). However, on day 17 (three days post-mixing) piglets mixed at 14 days of age had significantly higher lesion scores than the, as yet, unmixed litters (2.58 vs. 1.21, s.e.d 0.295, $P<0.001$). A similar increase was observed three days post-weaning (day 27) when piglets mixed at weaning had significantly increased lesion scores compared with piglets mixed at 14 days of age (4.08 vs. 2.64, s.e.d 0.357, $P<0.001$) and remained significantly higher (1.51 vs. 1.09, s.e.d 0.180, $P=0.025$ for day 33) until day 36 when it returned to a similar level as piglets previously mixed at 14 days.

Piglets relocated at weaning had significantly higher lesion scores compared to piglets left in the farrowing room on day 33 (1.50 vs. 1.10, s.e.d 0.180, $P=0.032$). On day 36 this had reversed and there was a trend for piglets relocated at weaning to have lower lesion scores compared with piglets remaining in the familiar environment of the farrowing room (0.99 vs. 1.26, s.e.d 0.152, $P=0.083$).

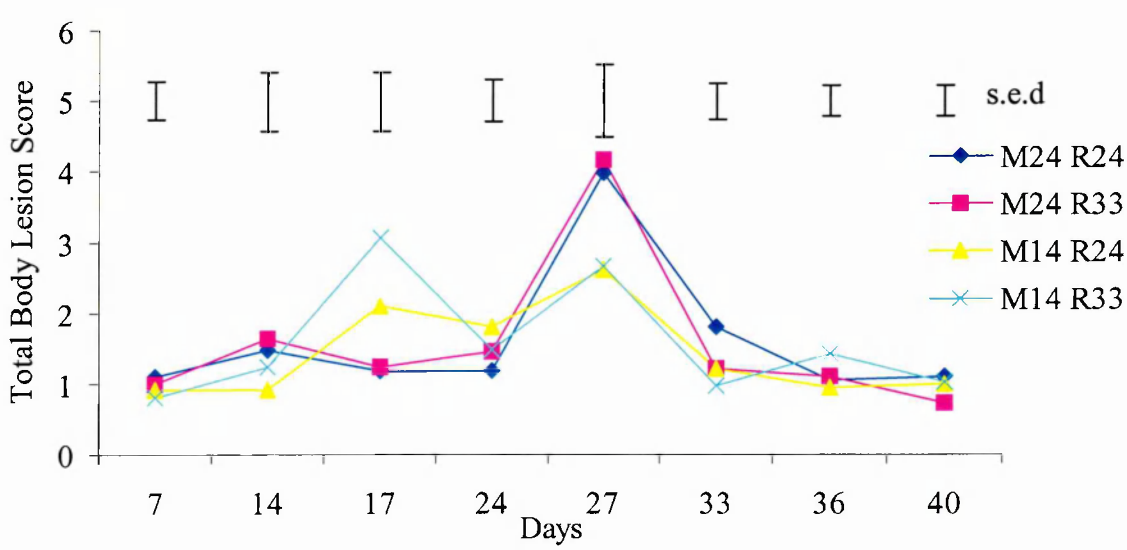

Figure 6.2 The effect of mixing piglets pre-weaning and relocation to a new environment at weaning on piglet total body lesion score

6.3.3 Behavioural observations

Teat orders were assessed pre- and post-mixing. It was observed that there was an increase in the average number of changes to teat order in the mixed group compared to the unmixed group (77% vs. 90%). These changes may have resulted from changes occurring when piglets are trying to suckle from their own mother but avoid any piglets from another sow interfering and so altering the teat positions of individual piglets within the litter. There were three incidences of cross-suckling observed after mixing but there was no significant effect of cross-suckling on piglet growth.

6.3.4 Humoral immune response

There was a significant mixing x relocation interaction on piglet anti-KLH IgG_1 antibody response on day 40 (Table 6.5 and Figure 6.3) where piglets mixed at 14 days of age and relocated at weaning to an unfamiliar environment had a significantly lower antibody response to KLH compared with all other treatment groups (P=0.002). On day 40, piglets mixed pre-weaning also had a significantly lower antibody response compared to piglets mixed at weaning (0.920 vs. 1.108(OD_{405nm}), P=0.010). Piglets that were relocated at weaning had significantly lower antibody responses compared with piglets which remained in the familiar environment for 9 days post-weaning on day 47 (0.786 vs. 1.012(OD_{405nm}), P<0.001).

Table 6.5 *Effect of mixing prior to weaning and time of relocation on piglet anti-KLH IgG_1 antibody response post-weaning (-log_{10} transformation)*

	Treatment					Significance		
	M24 R24	M24 R33	M14 R24	M14 R33	s.e.d	mix	relocate	mix x relocate
Anti-KLH IgG_1 Response (OD_{405nm}) (untransformed means)								
Day 24	1.524 (0.186)	1.667 (0.027)	2.139 (0.012)	1.597 (0.031)	0.1822	-	-	-
Day 40†	-0.068 (1.208)	0.013 (1.008)	0.140 (0.838)	0.001 (1.001)	0.0476	0.010	NS	0.002
Day 47†	0.111 (0.815)	0.018 (0.995)	0.171 (0.758)	-0.008 (1.029)	0.0481	NS	<0.001	NS

† Means adjusted for day 24 as a covariate

Anti-KLH IgG_2 antibody response to KLH gave a similar significant mixing x relocation interaction to IgG_1 on day 40 (Table 6.6 and Figure 6.4) with piglets mixed at 14 days and relocated at weaning having a significantly lower antibody response to the other treatments (P=0.002). There was no significant effect of mixing at any time, however piglets relocated to an unfamiliar environment at weaning had a continuously lower antibody response to KLH from day 40 through to day 47 (0.359 vs. 0.461(OD_{405nm}), P=0.034 and 0.315 vs. 0.441(OD_{405nm}), P=0.017 for day 40 and 47 respectively).

Table 6.6 *Effect of mixing prior to weaning and time of relocation on piglet Anti-KLH IgG_2 antibody response post-weaning (-log_{10} transformation)*

	Treatment					Significance		
	M24 R24	M24 R33	M14 R24	M14 R33	s.e.d	mix	relocate	mix x relocate
Anti-KLH IgG_2 Response (OD_{405nm}) (untransformed means)								
Day 24	1.365 (0.120)	1.399 (0.061)	1.950 (0.016)	1.254 (0.117)	0.1997	-	-	-
Day 40†	0.338 (0.449)	0.421 (0.421)	0.560 (0.269)	0.330 (0.500)	0.0579	NS	0.034	0.002
Day 47†	0.484 (0.374)	0.432 (0.418)	0.581 (0.256)	0.387 (0.465)	0.0683	NS	0.017	NS

† Means adjusted for day 24 as a covariate

Piglets that were mixed at weaning and remained in the farrowing room post-weaning had a significantly lower anti-KLH IgM antibody response compared with the other treatments on day 40 (P=0.037) (Table 6.7 and Figure 6.5). A significant effect of mixing was observed on day 47 with piglets mixed prior to weaning at 14 days of age had higher anti-KLH IgM response compared with piglets mixed at weaning (0.321 vs. 0.267(OD_{405nm}), P=0.007). There was no significant effect of relocation on anti-KLH IgM levels although a tendency was observed at day 40 where piglets relocated at weaning tended to have a higher anti-KLH IgM level than piglets left in the familiar environment of the farrowing rooms (0.315 vs. 0.282(OD_{405nm}), P=0.070).

Table 6.7 *Effect of mixing prior to weaning and time of relocation on piglet Anti-KLH IgM antibody response post-weaning (-log_{10} transformation)*

	Treatment					Significance		
	M24 R24	M24 R33	M14 R24	M14 R33	s.e.d	mix	relocate	mix x relocate
Anti-KLH IgM Response (OD_{405nm}) (untransformed means)								
Day 24	1.577 (0.046)	1.629 (0.032)	1.700 (0.023)	1.658 (0.034)	0.1303	-	-	-
Day 40†	0.511 (0.321)	0.614 (0.249)	0.526 (0.309)	0.519 (0.316)	0.0367	NS	NS	0.037
Day 47†	0.610 (0.247)	0.552 (0.287)	0.497 (0.328)	0.524 (0.314)	0.0360	0.007	NS	NS

† Means adjusted for day 24 as a covariate

Piglets mixed at weaning and left in the farrowing room post-weaning had significantly lower anti-KLH IgA levels compared to the other treatments and piglets mixed at 14 days and left in the farrowing rooms also had significantly lower anti-KLH IgA antibody response compared with piglets moved to an unfamiliar environment and either mixed before or at weaning (P=0.001) on day 40 (Table 6.8) but by day 47 there was no significant mixing x relocation interaction (Figure 6.6).

Piglets mixed at 14 days of age had a significantly higher level of anti-KLH IgA response compared with piglets mixed at weaning on day 47 (0.185 vs. 0.153(OD_{405nm}), P=0.004).

Table 6.8 *Effect of mixing prior to weaning and time of relocation on piglet Anti-KLH IgA antibody response post-weaning (-log_{10} transformation)*

	Treatment					Significance		
	M24 R24	M24 R33	M14 R24	M14 R33	s.e.d	mix	relocate	mix x relocate
Anti-KLH IgA Response (OD_{405nm}) (untransformed means)								
Day 24	1.852 (0.005)	2.318 (0.007)	1.506 (0.005)	2.439 (-0.012)	0.1315	-	-	-
Day 40[†]	0.726 (0.184)	0.635 (0.181)	0.889 (0.174)	0.455 (0.249)	0.0801	NS	NS	0.001
Day 47[†]	0.912 (0.129)	0.754 (0.177)	0.874 (0.155)	0.563 (0.214)	0.0939	0.004	NS	NS

[†] Means adjusted for day 24 as a covariate

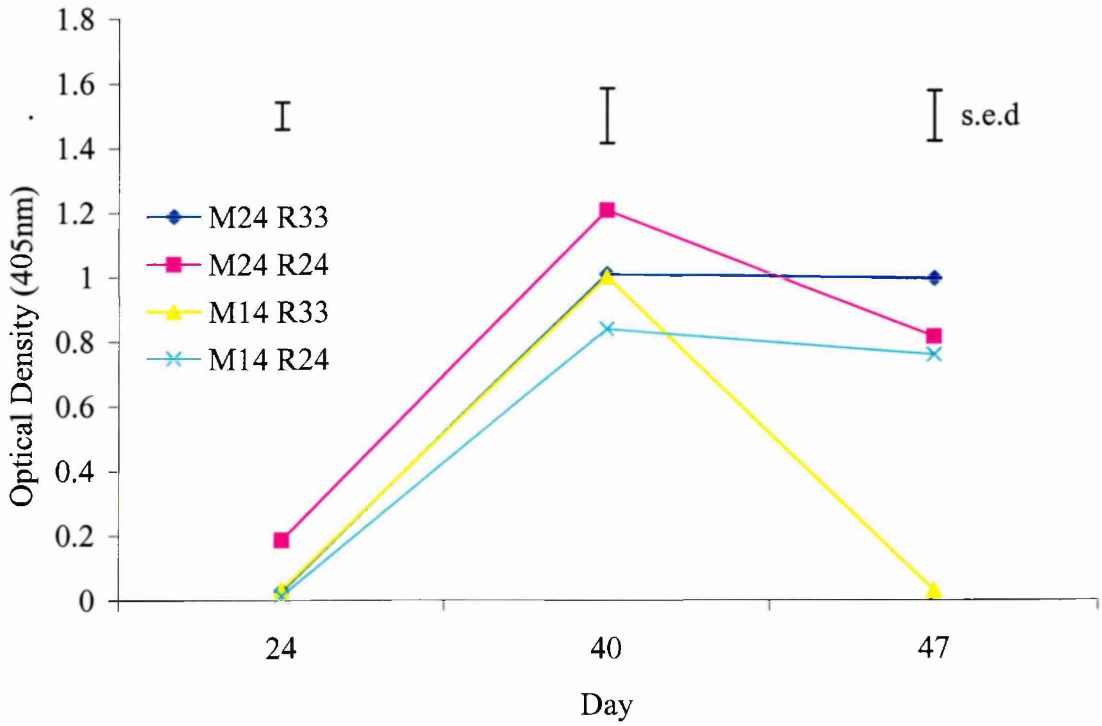

Figure 6.3 Effect of mixing pre-weaning and relocation on Anti-KLH IgG_1 antibody response

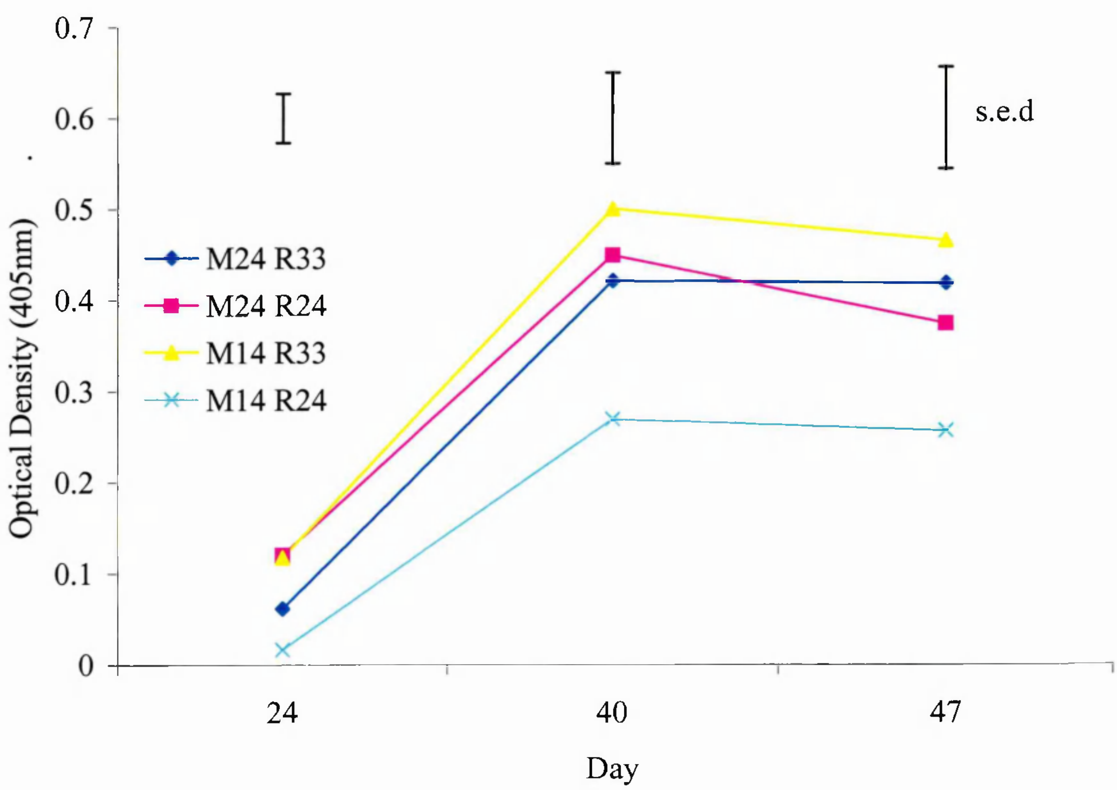

Figure 6.4 Effect of mixing pre-weaning and creep feed availability on Anti-KLH IgG_2 antibody response

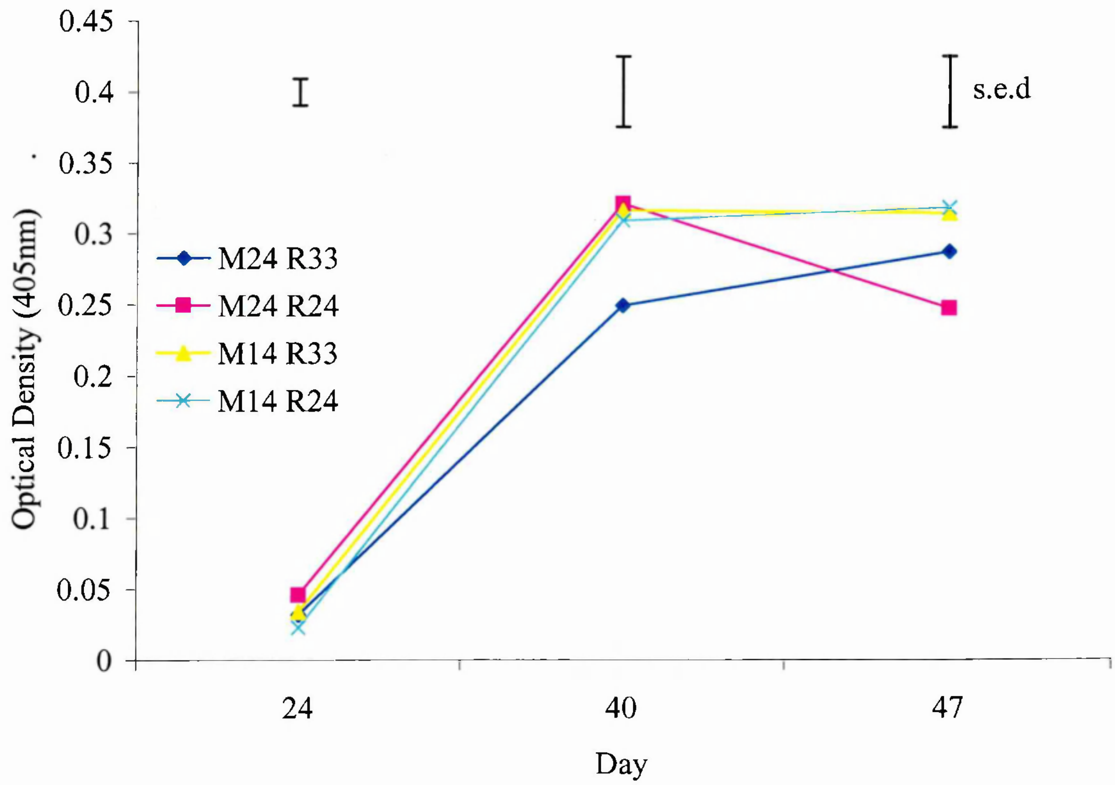

Figure 6.5 Effect of mixing pre-weaning and creep feed availability on Anti-KLH IgM antibody response

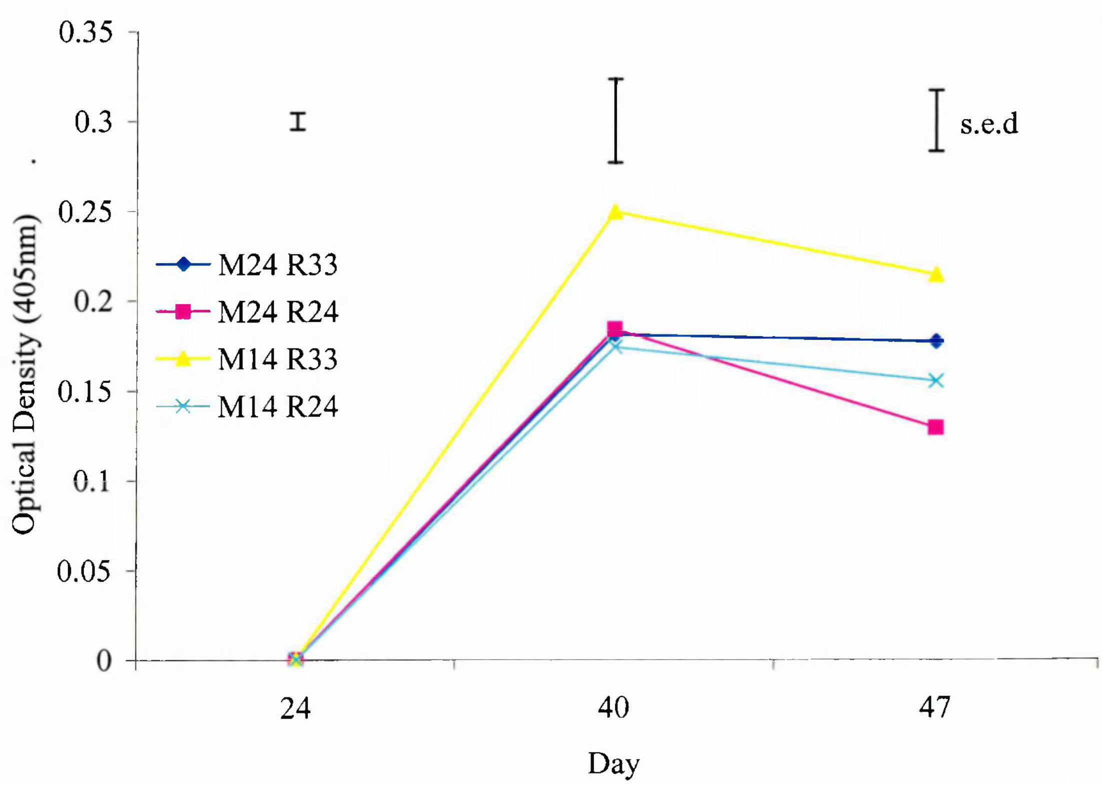

Figure 6.6 Effect of mixing pre-weaning and creep feed availability on Anti-KLH IgA antibody response

6.3.5 Gut morphology

Villus height and crypt depth was measured at 25, 50 and 75% along the length of the small intestine starting at the beginning of the duodenum (Table 6.9). No significant effects of treatment were observed at any distance along the small intestine. However, there was a tendency at 75% along the small intestine where piglets relocated into an unfamiliar environment at weaning appeared to have a decreased villus height compared to piglets remaining in the familiar environment of the farrowing room (200μm vs. 254μm, s.e.d 17.4, *P*=0.054). There was also a tendency for sections taken at 50% along the small intestine from piglets mixed at 14 days of age to have deeper crypts than sections from piglets not mixed until weaning (267μm vs. 187μm, s.e.d 39.4, *P*=0.089).

Table 6.9 *Effect of mixing and relocation on piglet villus height and crypt depth 7 days post-weaning*

	Treatment					Significance		
	M24 R24	M24 R33	M14 R24	M14 R33	s.e.d	Mix	relocate	Mix x relocate
Villus height (μm)								
25%	301	287	332	284	52.7	NS	NS	NS
50%	293	276	306	408	53.1	NS	NS	NS
75%	199	251	201	257	24.5	NS	NS	NS
Crypt depth (μm)								
25%	198	216	188	281	47.6	NS	NS	NS
50%	171	204	318	217	55.7	NS	NS	NS
75%	171	121	152	125	29.8	NS	NS	NS
Villus height:Crypt depth ratio								
25%	1.60	1.67	1.71	1.24	0.216	NS	NS	NS
50%	1.79	1.48	1.38	1.61	0.199	NS	NS	NS
75%	1.65	2.22	1.76	1.85	0.274	NS	NS	NS

6.3.6 Adrenal glands

Adrenal glands were removed from piglets after euthanasia and weighed to assess differences in previous adrenal activity (Hessing *et al.*, 1994). Adrenal glands were compared as a proportion of the piglets final body weight (Table 6.10). There was no significant interaction or effect of relocation on adrenal gland weight. Piglets mixed at 14 days of age had significantly heavier right adrenal glands compared with piglets mixed at weaning (0.066 vs. 0.049, s.e.d 0.0066, *P*=0.031). There was also a tendency for the left adrenal gland in piglets mixed at 14 days of age to also be heavier compared with piglets mixed at weaning (0.062 vs. 0.051, s.e.d 0.0056, *P*=0.087).

Table 6.10 *Effect of mixing and relocation on piglet adrenal gland weight 7 days post-weaning*

	Treatment					Significance		
	M24 R24	M24 R33	M14 R24	M14 R33	s.e.d	Mix	relocate	Mix x relocate
Adrenal gland weight (Proportion of final body weight)								
Left	0.054	0.048	0.058	0.065	0.0080	NS	NS	NS
Right	0.055	0.044	0.066	0.067	0.0093	0.031	NS	NS

6.4 DISCUSSION

As previous chapters have discussed the main effect of mixing on piglet performance and immune status mixing will only be discussed briefly and when there appears to have been an interactive effect with relocation.

6.4.1 Performance

In general terms, the improvements previously observed when mixing pre-weaning occurred were absent from this trial. Again this may have been due to the weaning age being different and therefore weaning was closer to the mixing of piglets and, as seen in chapter 3, mixing too close to weaning appeared to have a detrimental effect on post-weaning growth.

In the period immediately following weaning considerable reductions in growth rates were seen in all treatments and these growth checks are approximately 50% greater than observed in chapters 3, 4 and 5. This can be attributed to weaning at 24 days of age compared to weaning at 28 days of age in previous trials. There have been a variety of studies of the effect of weaning age on performance (Bøe, 1993, Worobec *et al.*, 1999) and piglets weaned earlier in their development are more likely to have decreased growth rates immediately post-weaning as a result of the inadequate development of the immune system and digestive tract. Worobec *et al.* (1999) compared weaning piglets at 7, 14 and 28 days of age and by six weeks of age those piglets weaned at 7 days were significant lighter than piglets weaned later. Their results suggested that weaning at 14 days of age or earlier might result in a reduction in performance and development of behavioural patterns.

Relocation to a novel environment has previously been reported to increase the motivation for exploration in piglets (Bøe, 1993). However, when the new environment is essentially barren, there is an increased chance that piglets redirect their motivation towards pen mates resulting in belly nosing and tail biting (Bøe, 1993). Yet this barren environment also creates a significantly greater chance of the piglets' discovering the alternative feed source provided in the absence of the sow. This reduces the time spent fasting by piglets post-weaning because of their inability to discover the new feed source (Makkink, 1993) and this may account for the improved feed intakes and growth rates observed in piglets relocated to the flat deck accommodation at weaning. Piglets remaining in the farrowing rooms post-weaning may have had a reduced motivation to explore their familiar environment after weaning and search for alternative food sources as they expected the sow to return to feed them. It is also possible that the increased feed intakes resulted from the piglet's boredom in the barren environment and that feed was the most interesting stimulus within the pen.

Piglets remaining in the familiar environment also had to contend with the effect of environmental temperature. The flat deck accommodation allowed strict control of the temperature of the room whereas the farrowing room was not as easily controlled, as illustrated by the temperature ranges of the rooms (section 6.2.1). Even though these temperature ranges are similar, if the temperature was not constant throughout the farrowing room, i.e. it was warmer in the lying area where additional heat lamps were provided, then piglets may be less inclined to leave the lying area to feed in a colder environment (Ingram, 1964). Close and Stanier (1984) identified that for piglets weaned at 21 days the lowest temperature to gain optimum growth rates was

23°C. However, these piglets were weaned at 21 days of age and as physiologically younger may have been less able to cope with the stress of weaning plus the additional cold stress of a change in temperature compared with piglets weaned at 24 days of age. Piglets weaned at 21 and 28 days of age into flat deck accommodation also had depressed growth, intake and FCR when temperatures were reduced 1 week post-weaning from 29°C to 24°C (McConnell *et al.*, 1987).

6.4.2 Lesion scores

Lesion scores observed throughout the trial are consistent with the effects reported in chapters 3, 4 and 5, in terms of increased lesion post-mixing and post-weaning regardless of the change in weaning age with similar levels of lesions being observed throughout the trial. However, there were no significant changes to lesion scores resulting from relocation. This may indicate that relocation does not significantly affect the aggressive interactions associated with weaning. However, it has previously been observed that piglets relocated to flat decks have more severe lesions especially on the tail compared with piglets remaining in the familiar environment (Bøe, 1993, Puppe *et al.*, 1997) although the previous studies have differed in weaning age with weaning occurring as late as 42 days (Puppe *et al.*, 1997). Tail lesions in this trial were largely absent with a few minor abrasions appearing on a few piglets. This could be attributed to the early age of weaning and therefore the size of the piglets. It is clear from the results from chapters 3, 4 and 5 that as the piglet increases in size then the severity of lesions also increases and therefore may explain the differences observed with relation to changes of environment.

6.4.3 Behavioural observations

There was no significant effect of mixing pre-weaning and relocation on teat orders although a positive correlation was observed between teat order and growth rate thus supporting previous studies (Hartstock *et al.*, 1977: Hoy and Puppe, 1992). Both studies reported that piglets suckling from the anterior teats had significantly higher growth rates compared with piglets suckling the middle and posterior teats. Dyck *et al.* (1987) suggested that the anterior teats were more productive and therefore growth rates were improved at these teats due to increased milk production. Jeppesen (1982) also suggests this although for a different reason, in that larger piglets tend to gain access to these teats and the larger piglets are better able to stimulate milk production due to their size.

A variety of observations have been made regarding the time spent performing different behaviours in a familiar environment versus a unfamiliar environment such as flat deck accommodation. Belly nosing (Metz and Gonyou, 1990) and tail biting (Bøe, 1993) were significantly increased in the unfamiliar environment but there were no significant lesions on the tails or belly of the piglets observed throughout this trial. This may possibly be due to the different weaning age ranges used of 2-4 weeks (Metz and Gonyou, 1990) and 4-6 weeks (Bøe, 1993) with piglets weaned early at 2 weeks exhibiting belly nosing while the larger piglets (6 weeks of age) exhibited tail biting more frequently. Overall very few stereotypic behaviours were observed during the post-weaning period regardless of environment. Further research is required to identify the effect of the temperature fluctuations within the farrowing room on piglet's motivation to move from the heatpads to find food and to adjust the temperature accordingly.

The clear reductions in feed intakes of piglets remaining in the familiar environment of the farrowing rooms possibly highlights the fact that by not relocating piglets at weaning the social stress of losing maternal contact is being prolonged as the piglets are waiting for the sow to return and this is therefore a negative stress. Wattanakul *et al.* (1998) reported that separating the piglets from the dam for up to 6 hours did not result in any extra intake in creep feed and could imply that maternal deprivation is more important than the change in environment. Therefore the change in location that usually occurs at weaning may be a positive stress that encourages piglets to forget the sow and get on and explore the new surroundings. This concurs with the observation by Jensen (1995) in natural conditions, piglets expect the sow to be absent for ever longer periods of time.

6.4.4 Immune responses

As previously reported in chapter 4, the stress of mixing at weaning can cause an increase in anti-KLH antibody response and these results from this experiment support these studies.

The effect of relocation on anti-KLH response is only significantly apparent for subclasses IgG_1 and IgG_2. If stress causes this increase in anti-KLH antibody response (Pollock *et al.*, 1992) then the results observed here of increased antibody levels post-weaning in piglets remaining within a familiar environment may indicate that piglets relocated immediately after weaning are coping with the adaptation of weaning better than piglets believed to be less stressed by not being relocated.

The increase in the level of stress and elevated immune response may have been caused by the fluctuating temperatures within the farrowing room and the piglets' desire to stay within their thermoneutral zone and huddle together rather than move to a colder environment to feed (Close *et al.*, 1971). However, this contradicts work by Crenshaw *et al.* (1986) where no effect of weaning or environmental temperature was observed on total antibody production when piglets were weaned at 2 or 3 weeks of age and maintained at environmental temperatures of 18°C or 25°C which are lower and similar, respectively, to the environmental temperatures of the farrowing room post-weaning. The measure of total antibody response is not specific and therefore there is less chance of seeing a response due to the different control mechanisms for the classes of antibody (Rook *et al.*, 1994). The use of a different antigen (foreign erythrocytes) would also have had an effect as an alum precipitated antigen, such as KLH, is processed differently from a membrane bound antigen, such as erythrocytes, hence stress may have caused different responses (Roitt *et al.*, 1998; Alexander and Brewer, 1995).

6.4.5 Gut morphology

The poor growth rates and decreased food intakes characteristic of post-weaning period are often related to the effect of weaning on the development of the digestive tract (Cera *et al.*, 1988; Funderburke and Seerley, 1990; Nabuurs *et al.*, 1993). The digestive tract of the piglet is relatively underdeveloped to cope with the dietary change enforced upon the piglet at weaning and combined with the stress of other weaning factors such as relocation and mixing (Kenworthy, 1976; Hampson, 1986b).

The reduced ability of the piglet to absorb the nutrients required is caused by the classic reduction in villus height observed due to the stress of weaning and dietary change (Kenworthy, 1976). The classic villus atrophy and crypt hyperplasia was observed across all treatment groups post-weaning compared with the unweaned controls of previous studies (Hampson, 1986a; Bruininx *et al.*, 2001). There was no apparent effect of mixing and relocation on feed intakes or feed conversion ratios and therefore it is difficult to draw any conclusions on the relationship between these performance parameters and gut morphology in this experiment. However, the lack of any effect on gut morphology could possibly be due to the main effect of weaning which has previously been reported to cause changes in gut morphology, i.e. dietary changes using a three phase feeding regime during the three week post-weaning period and this needs to be investigated further.

6.4.6 Adrenal glands

Adrenal gland weight has been shown to reflect cortisol production in piglets (Hessing *et al.*, 1994; Manning and Stamp Dawkins, 1998) and therefore may be used to assess the long-term activation of the hypothalamus-pituitary adrenal (HPA) axis and used as an indicator of welfare (Hessing *et al.*, 1994). Beattie *et al.* (2000) observed an increase in adrenal weight in pigs relocated to a barren environment, where as there was no significant change in adrenal gland weight due to relocation during this trial. This may have been the result of the relative short period of this trial (relocation only occurred 7 days prior to slaughter) compared with the 21 weeks carried out in the barren versus enriched environments reported by Beattie *et al.* (2000).

Mixing pre-weaning appeared to cause an increase in adrenal weight suggesting that mixing piglets prior to weaning may have caused increased activation of the HPA axis in the long-term and, possibly, a reduction in piglet's welfare. However, this was not reflected in the performance of those piglets mixed at 14 days of age but further investigation of this increased adrenal weight must be considered before any final conclusions of mixing can be drawn.

6.5 CONCLUSIONS

This study aimed to assess the effect of keeping piglets in a familiar environment on the disruption of growth usually seen when weaning occurs at 3-4 weeks. However, it appears that remaining in a familiar environment is more disruptive to piglet growth than relocation at weaning to unfamiliar flat deck accommodation and that weaning at 24 days of age compared to 28 days reduced the improvements in growth previously observed when mixing piglets pre-weaning. There also appears to be a learning trigger at weaning between the change in environment and separation from the sow which initiates the piglet to start searching for alternative food sources and therefore relocation to a new environment may be a positive stress.

Further investigations of digestive tract morphology regarding post-weaning performance and feed intakes along with more research into the effect of mixing on welfare as indicated by the increased adrenal weights need to be carried out to fully assess the optimum weaning management strategy.

CHAPTER 7. THE EFFECT OF MIXING PRE-WEANING AND EARLY CREEP FEED AVILABILITY ON PIGLET PERFORMANCE BEHAVIOUR, IMMUNE FUNCTION AND GUT MORPHOLOGY

7.1 INTRODUCTION

It has been shown previously that mixing prior to weaning can have significant benefits to piglet's performance post-weaning (chapters 3 & 4) and that a change of environment at weaning also appears to be beneficial to the piglet's performance post-weaning (chapter 6). The nutrient requirements of the newly weaned piglet have been well documented and it is clear that a good quality diet which is highly palatable is essential for the piglet during this stressful period (Thacker, 1999).

The provision of creep feed prior to weaning has undergone extensive research but with inconsistent results (Okai *et al.*, 1976; Waran and Broom, 1992; Fraser *et al.*, 1994). Appleby *et al.* (1991) identified that cleaning and refilling the hoppers with feed provoked exploratory behaviour and increased feeding frequency. It was also noted that increased access to creep significantly improved feed intake and this may be connected to social facilitation and the imitation of other piglets eating creep feed (Appleby *et al.*, 1991). These findings were consistent with those of Kavanagh (1996) and Delumeau and Meunier-Salaun (1995).

As identified in chapter 1, creep feed intakes have been found to be highly variable between and within litters ranging from 0-2382g/litter and 0-674g/piglet (Delumeau and Meunier-Salaun, 1995) prior to weaning. Pajor *et al.* (1991) showed that total creep consumption varied from 13-1911g/piglet from 2 weeks of age to weaning at 4 weeks and suggested that higher creep feed intakes were typical of larger, more mature piglets than as a compensation for poor milk intake. It has also been identified that female piglets eat more creep than males (Waran and Broom, 1992; Delumeau and Meunier-Salaun, 1995).

Piglets have been reported to choose an appropriate diet by selecting feeds that were high or low in protein relevant to the size or dietary requirements of the individuals (Kyriazakis *et al.*, 1990; Dalby *et al.*, 1995). Therefore, it may be possible to use this technique to allow piglets to move onto the next stage diet once they reach the required size and thus reduce the need to feed for the smallest piglet within a large group of piglets.

Having reported the improvements in piglet performance post-weaning associated with mixing pre-weaning this element of altering weaning management strategies was utilised in conjunction with the creep feeding elements to assess any interactions between mixing and creep feed consumption. Previously it has been observed, although not at significant levels, that mixed piglets appear to consume more creep in the last week prior to weaning. It is essential to identify the social factors that influence creep feed intake to enable improved management of creep feeding. The aim of this study was to examine the effects of access to creep feed from 6 days of age on piglet performance, gut morphology, immune status and post-weaning feeding behaviour. It also aimed to assess the interaction between mixing prior to weaning on creep intake and access to creep feed.

7.2 MATERIAL AND METHODS

7.2.1 Animals and Housing

Forty-eight sows (40 Camborough 15 sows (Large White x (Landrace x Duroc)) and 8 homebreds as discussed in section 2.1) and their 453 piglets kept in conventional farrowing pens (section 2.1) were randomly allocated to a 2 x 2 factorial experimental design (Figure 7.1). Four replicates were carried out and each treatment contained three sows and their piglets therefore using 12 sows per replicate.

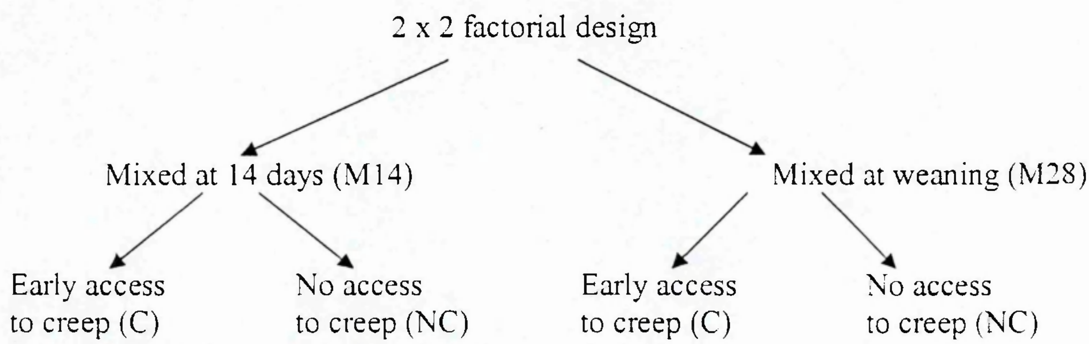

Figure 7.1 Experimental design for mixing prior to weaning and early access to creep in a 2 x 2 factorial design

Mixing of piglets was carried out by the removal of the boards in between each pen as described in section 3.1 and standard management procedures were carried out within 24 hours (section 2.1). Weaning took place at 28 days when all piglets were moved from the farrowing pens into flat deck accommodation (section 2.1 & Figure 7.2). All piglets remained in their specific treatment groups and housed in one large pen.

Figure 7.2 Piglets mixed at 14 days of age in the new flat deck accommodation

7.2.2 Creep feed management

Creep feed (Target 2, Ian Hollows Diets) was supplied to piglets in the relevant treatments from 6 days onwards in a creep bowl. During each feeding session, creep bowls were placed in the pens and removed and creep weighed back. Fresh creep feed was supplied using a three-stage regime as follows:

Day 6-13: Creep provided for one-hour morning and afternoon.

Day 14-20: Creep provided for one hour in the morning and three hours in the afternoon.

Day 21-28: Piglets had continuous access to creep except for 30 minutes in the morning and evening and one hour at midday.

Appleby *et al.* (1991) reported that cleaning and refilling the feeders stimulated piglet's exploratory behaviour and increased creep consumption therefore this method was adapted to maintain the piglet's interest in the creep feed provided to them.

7.2.3 Performance

Piglets were weighed within 24 hours of birth (day 0), then on days 7, 14 (day of mixing), 17, 21, 28 (weaning), 31, 35, 42, 49, 56 according to the standard operating procedure (section 2.1). Post-weaning, feed was supplied *ad libitum* into four troughs and was weighed back at each piglet weighing to allow feed intakes to be calculated. For the first seven days post-weaning all piglets were offered the same diet previously given as creep feed prior to weaning.

From seven days post-weaning onwards each treatment group had access to a choice of two commercial diets each week. This choice feeding allowed the piglets to select the diet that was most appropriate to them according to Dalby *et al.* (1995). The feeding regime used four commercial weaner diets and a commercial grower feed using the following schedule:

Day 28-35: Piglets had access to Ian Hollows Target 2.

Day 36-42: Piglets had access to Ian Hollows Target 2 and Target 3.

Day 43-49: Piglets had access to Ian Hollows Target 3 and Target 4.

Day 50-56: Piglets had access to Ian Hollows Target 4 and Bibby's Grower diet.

Table 7.1 shows proximate analysis for all of the feeds.

Table 7.1 *Proximate analysis of diets*

	Target 2	Target 3	Target 4	Grower
Proximate Analysis				
Dry Matter (g/kg)	894	882	877	869
Crude Protein (g/kg DM)	249	253	262	240
Oil (g/kg DM)	79	50	51	45
Ash (g/kg DM)	70	65	64	50
NDF (g/kg DM)	163	159	187	170
DE (MJ/kg DM)†	16.1	15.9	15.6	16.0

† DE = 17.47 + 0.0079CP + 0.0158Oil – 0.0331Ash – 0.0140NDF
(CP, Oil, Ash and NDF in g/kg DM) based on calculation by MAFF (1993)

7.2.4 Lesion scoring

Each piglet was examined for lesions at each weighing according to the method described in section 2.2.2. Each section was scored for fresh lesions only and classified accordingly (Table 2.1)

7.2.5 Behavioural observations

Teat order was assessed before mixing occurred and again on days 1 and 7 post-mixing according to the method outlined in section 2.2.4.3. Cross-suckling of the sows by the piglets was recorded during the teat order observation period post-mixing.

Creep feeding behaviour was observed to identify which piglets were eating the creep. Each piglet was given an individual identification mark and then the pens were recorded with time lapse video equipment on day 24 to identify which piglets were spending more time at the creep bowls over a 24 hour period and general feeding behaviour patterns. Feeding behaviour post-weaning was also recorded over a 24-hour period on days 37 and 38 to look at the effect of offering two diets on feeding behaviour and identify which piglets were eating which diet.

7.2.6 Immune responses

Blood samples were collected from 96 piglets (four randomly selected piglets/litter) by venipuncture in the supine position at weaning (day 28) and on days 35, 42 and 49 as described in section 2.2.3.

7.2.6.1 Humoral immune response

Humoral immune responses were assessed by immunising the piglets at weaning with 1mg/ml KLH (alum precipitated) (section 2.4.1) at weaning and assessing serum anti-KLH antibody responses using an ELISA based on the method by Pollock *et al.* (1991) described in section 2.4.2.

7.2.6.2 Cell mediated immune response

Cell-mediated immune responses were assessed on isolated peripheral blood mononuclear cells (section 2.4.4) using the lymphocyte blastogenesis MTT assays as described in section 2.4.5.2.

7.2.6.3 IFN–γ Assay

Gamma interferon production by peripheral blood mononuclear cells were assessed on day 35 using a 48 hours incubation followed by the IFN–γ ELISA on the culture supernatant as described in section 2.4.6.

7.2.7 Gut morphology

One piglet from each litter was slaughtered on day 33 (5 days post-weaning) and the small intestine dissected out as described in section 2.2.5. This day was chosen, as it has previously been reported as being the optimum time to see villus atrophy compared with non-weaned piglets of the same age (Hampson, 1986b; Pluske *et al.*, 1991). The length of the small intestine was measured and then three sections selected at 25%, 50 % and 75% along the tract were removed and stored in 10% buffered formalin for analysis of crypt depth and villus height as described in section 2.5.1. The small intestine was then washed out and weighed to give an empty intestine weight.

7.2.8 Adrenal glands

The adrenal glands were also removed post-euthanasia on day 33 and any additional fat was trimmed before being weighed (section 2.2.5).

7.2.9 Statistical analysis

Statistical analysis was performed using Genstat for Windows Version 4.1. Live weights were analysed using repeated measures analysis of variance and Antedependence Modelling. Analysis of variance was performed on all other performance data. Treatment means were compared using a Protected Least Significant Difference (Snedecor and Cochran, 1993). Statistical significance was accepted at $P<0.05$. In the farrowing house, the sow and her litter were identified as the experimental unit (d.f. = 41) but in the flat deck the whole pen (i.e. treatment) was used as the experimental unit (d.f. = 9).

7.3 RESULTS

7.3.1 Performance

There was no difference between the treatments in the number or age of piglets at any stage throughout the study. There was also no significant effect of treatment on piglet mortality (4%) or morbidity (35%). There was an increased level of morbidity due to two cases of scours in two of the replicates of the trial. One case in the farrowing room affected seven litters and a second case due to a draught in the flat deck accommodation affected 50% of the piglets.

Repeated measures analysis of variance of live weights showed no significant treatment x time interaction. However further analysis of live weight using antedependence modelling determined that an order 2 model was required therefore the previous two weights were used as covariates in the analysis of variance (Table 7.2).

Table 7.2 *Effect of mixing and access to creep on piglet live weights*

	Treatment					Significance		
	M28 C	M28 NC	M14 C	M14 NC	s.e.d	mix	creep	mix x creep
Live weight (kg)[†]								
Birth	1.58	1.63	1.59	1.53	0.091	NS	NS	NS
Day 7	2.61	2.78	2.72	2.91	0.182	NS	NS	NS
Day 14	4.67	4.60	4.56	4.45	0.118	NS	NS	NS
Day 17	5.36	5.30	2.30	5.34	0.051	NS	NS	NS
Day 21	6.46	6.34	6.40	6.52	0.113	NS	NS	NS
Day 28	8.30	8.45	8.22	7.98	0.153	0.017	NS	NS
Day 31	8.66	8.80	8.64	8.65	0.188	NS	NS	NS
Day 35	9.69	9.82	9.63	9.85	0.129	NS	NS	NS
Day 42	12.61	12.07	12.06	12.30	0.199	NS	NS	0.007
Day 49	15.62	15.72	15.76	15.56	0.215	NS	NS	NS
Day 56	19.55	19.25	19.12	19.46	0.233	NS	NS	NS

[†] Means adjusted for previous two weights as covariates according to antedependence model order 2

Piglets mixed at weaning and given access to creep feed were significantly heavier on day 42 compared with all other treatments (*P*=0.007) and there was a tendency for those piglets to still be heavier on day 56 (*P*=0.061). There was no significant effect of creep feed on piglet live weight at any time during the trial, however piglets mixed at 14 days of age were significantly lighter at weaning (day 28) compared with piglets remaining unmixed until after weaning (8.10kg vs. 8.37, s.e.d 0.108, *P*=0.017).

From day 36 – 42, piglets mixed at weaning and given creep were growing significantly faster than piglets mixed at weaning without creep and piglets mixed at 14 days of age with access to creep (Table 7.3). Piglets mixed at 14 days of age without creep were also growing significantly faster than piglets with access to creep mixed at either age (*P*=0.004). This difference was also apparent from day 50 to 56 although it was no longer significant (*P*=0.093).

Table 7.3 *Effect of mixing and creep access on piglet daily live weight gain*

	Treatment					Significance		
	M28 C	M28 NC	M14 C	M14 NC	s.e.d	mix	creep	mix x creep
DLWG (g/day)								
0-7†	161	187	174	174	17.5	NS	NS	NS
8-14†	262	259	248	234	21.9	NS	NS	NS
15-17†	273	249	236	253	17.5	NS	NS	NS
18-21†	266	252	268	288	27.6	NS	NS	NS
22-28†	268	265	253	229	19.5	NS	NS	NS
29-31‡	101	114	119	62	38.9	NS	NS	NS
32-35‡	246	274	233	268	32.5	NS	NS	NS
36-42‡	401	340	311	368	27.3	NS	NS	0.004
43-49‡	497	492	464	475	33.7	NS	NS	NS
50-56‡	555	519	484	538	37.0	NS	NS	NS
0-28†	244	250	236	233	13.4	NS	NS	NS
29-56‡	413	391	365	392	25.6	NS	NS	NS
0-56†	332	326	302	318	14.9	NS	NS	NS

† Means adjusted for birth weight as a covariate ‡ Means adjusted for weaning weight as a covariate

There was no significant effect of mixing or creep feed access on piglet daily live weight gain throughout the trial. Although there was a tendency for piglets mixed prior to weaning at 14 days to be growing slower than piglets mixed at weaning on days 22-28 (241g/day vs. 267, s.e.d 13.8, *P*=0.069) and also over the whole trial period (310g/day vs. 329, s.e.d 10.6, *P*=0.078). From day 36 to 42, piglets mixed at weaning and given creep feed had a significantly lower feed conversion ratio compared with the other treatments (*P*=0.027) (Table 7.4).

Table 7.4 *Effect of mixing and access to creep on creep intake, post-weaning feed intakes and feed conversion ratios*

	Treatment					Significance		
	M28 C	M28 NC	M14 C	M14 NC	s.e.d	Mix	Creep	mix x creep
Average creep intakes (g/pig/day)								
6-13	20	-	13	-	3.0	NS	-	-
14-20	73	-	79	-	5.1	NS	-	-
21-28	261	-	214	-	32.6	NS	-	-
6-28	354	-	305	-	37.8	NS	-	-
Average feed intakes (g/pig/day)								
28-31	141	140	146	107	27.8	NS	NS	NS
32-35	278	281	267	259	33.8	NS	NS	NS
36-42	445	410	381	441	33.3	NS	NS	NS
43-49	664	650	618	644	43.0	NS	NS	NS
50-56	863	825	790	862	50.2	NS	NS	NS
FCR								
28-31	2.33	3.75	1.20	0.03	2.640	NS	NS	NS
32-35	1.24	0.10	1.23	1.06	0.159	NS	NS	NS
36-42	1.16	1.27	1.31	1.25	0.045	NS	NS	0.027
43-49	1.34	1.35	1.29	1.38	0.071	NS	NS	NS
50-56	1.59	1.62	1.63	1.62	0.104	NS	NS	NS

Although individual feed intakes could not be measured during this trial, it can be calculated how much of each of the diets offered post-weaning during choice feeding was consumed each week (Table 7.5). It can be seen that there are no significant differences between the treatments at any stage during the trial except for a significant mixing x creep interaction for the amount of target 3 (*P*=0.004).

Table 7.5 *Effect of mixing and creep intake on the feed intakes of the diets offered for choice feeding post-weaning*

	Treatment					Significance		
	M28 C	M28 NC	M14 C	M14 NC	s.e.d	Mix	Creep	mix x creep
Average feed intakes (g/pig/day)								
Target 2	170	207	198	153	32.0	NS	NS	NS
Target 3	287	203	182	277	32.8	NS	NS	0.004
Average feed intakes (g/pig/day)								
Target 3	256	251	286	201	73.4	NS	NS	NS
Target 4	425	399	332	426	74.8	NS	NS	NS
Average feed intakes (g/pig/day)								
Target 4	731	740	706	785	48.8	NS	NS	NS
Grower	154	85	84	56	45.8	NS	NS	NS

Generally, it can be seen that piglets did show preferences between the two feeds offered and even though there are no differences between the treatments this could have been due to the limited differences between the weight of the treatments post-weaning, therefore all the piglets are selecting the food most appropriate to their weight and feed requirements. However, in the last week where a choice of target 4 and grower was offered there was a clear preference for the target 4 even though the piglets should have been of a correct weight to move onto the grower diet (Table 7.5).

Piglets mixed at weaning and offered creep and piglets mixed at 14 days of age but without access to creep showed a clear preference for the more palatable and digestible food in each of the stages when a choice was offered. All treatments show a similar pattern of feed choice when offered the last stage of the weaner diets (Target 4) and the grower diets with very small amounts of the grower diet being consumed (<100g/day). It was not possible to assess how the feed choice changed within each week they were provided but when the total amount of each diet consumed was calculated the proportions of each diet consumed were similar to those recommended commercially and provided in chapters 3,4,5 and 6.

7.3.2 Lesion scores

No significant mixing x creep interaction was observed at any time during the trial on piglet total body lesion score (Figure 7.3). There was also no significant effect of creep feed access on total body lesion score although there was a tendency for piglets given access to creep to have higher lesion scores compared with piglets not offered any creep on day 14 (1.43 vs. 0.81, s.e.d 0.347, P=0.079).

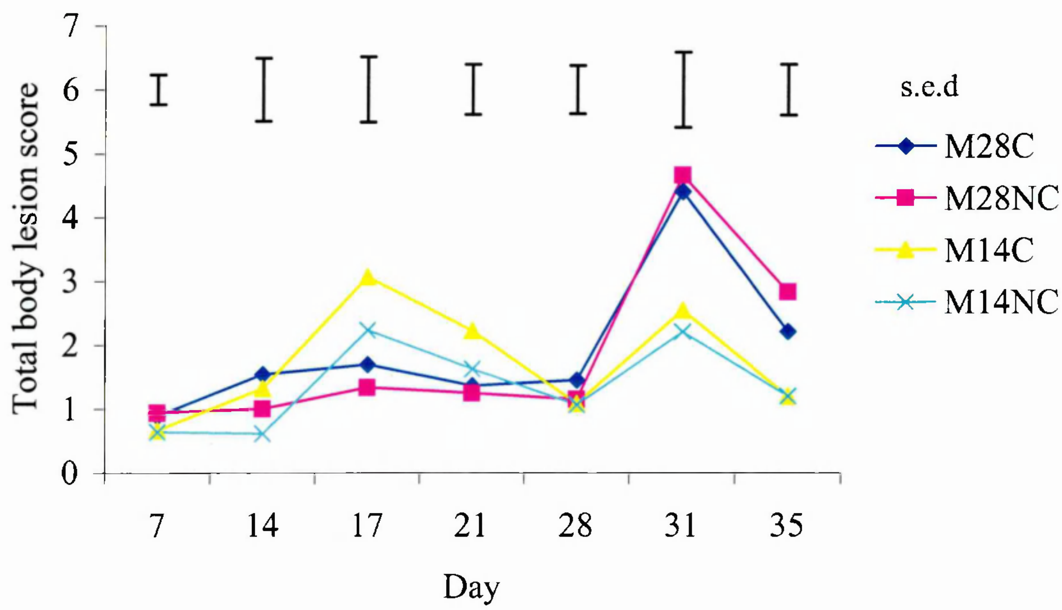

Figure 7.3 Effect of mixing and access to creep feed on total body lesion score.

Piglets mixed at 14 days of age had significantly higher lesion scores compared with piglets not mixed until weaning on day 17 (2.65 vs. 1.51, s.e.d 0.362, P=0.003) and 21 (1.93 vs. 1.30, s.e.d 0.276, P=0.027). However by day 31 piglets mixed at 14 days of age had significantly lower lesion scores compared with piglets mixed at weaning (2.37 vs. 4.53, s.e.d 0.418, P<0.001) and this significantly lower lesion score was still evident on day 35 (1.20 vs. 2.52, s.e.d 0.280, P<0.001).

7.3.3 Behavioural observations

Behaviour observations were carried out using time-lapse video recorders to assess pre-weaning creep feeding activity and post-weaning feed choice.

7.3.3.1 Pre-weaning behaviour observations

Prior to weaning comparisons of average feeding duration and frequency of visits to the creep trough between the mixed and unmixed groups were studied. The relationship between creep feed behaviour and position in the teat order was also assessed. There was a significant effect of mixing on the average duration of visits to the creep trough over a 24 hour period on day 24 (Table 7.6) with piglet mixed pre-weaning spent shorter times at the trough ($P<0.001$). However, this does not seem to have had an effect on the intake as shown in Table 7.4.

Table 7.6 *Effect of mixing prior to weaning on frequency of visits to the creep feed trough and the average duration of visits over a 24-hour period on day 24*

	Treatment			
	Mixed	Unmixed	s.e.d	Significance
Creep feed behaviour				
Frequency of visits	13.8	13.2	1.96	NS
Mean duration of visits	35.0	68.6	7.83	<0.001

There was also a significant effect of position in the teat order on frequency of visit to the trough (Table 7.7) with piglets suckling from the middle teats visiting the trough significantly more often than piglets suckling from the anterior or posterior teats ($P=0.008$). However, there was no significant effect on the duration of visits.

Table 7.7 *Effect of position in the teat order on frequency of visits to the creep feed trough and the average duration of visits over a 24-hour period*

	Teat order position				
	Anterior	Middle	Posterior	s.e.d	Significance
Creep feed behaviour					
Frequency of visits	11.2	17.6	11.1	2.60	0.008
Mean duration of visits	53.9	51.5	50.6	10.35	NS

7.3.3.2 Post-weaning choice feeding behaviour

The effect of offering two diets was observed over a 24-hour period on days 46 & 47 when the feeds Target 3 and Target 4 were being offered. There was a large variation between the patterns and frequency of visits of the different treatments to the different feed types over a 24 hour period (Figures 7.4, 7.5, 7.6 and 7.7). It is clear that there is difference between the number of visits to the different feeds and this supports the feed intakes observed for the treatments with most piglets eating Target 4 from day 42-49. All of the treatments show a clear diurnal pattern of feeding regardless of the feed chosen.

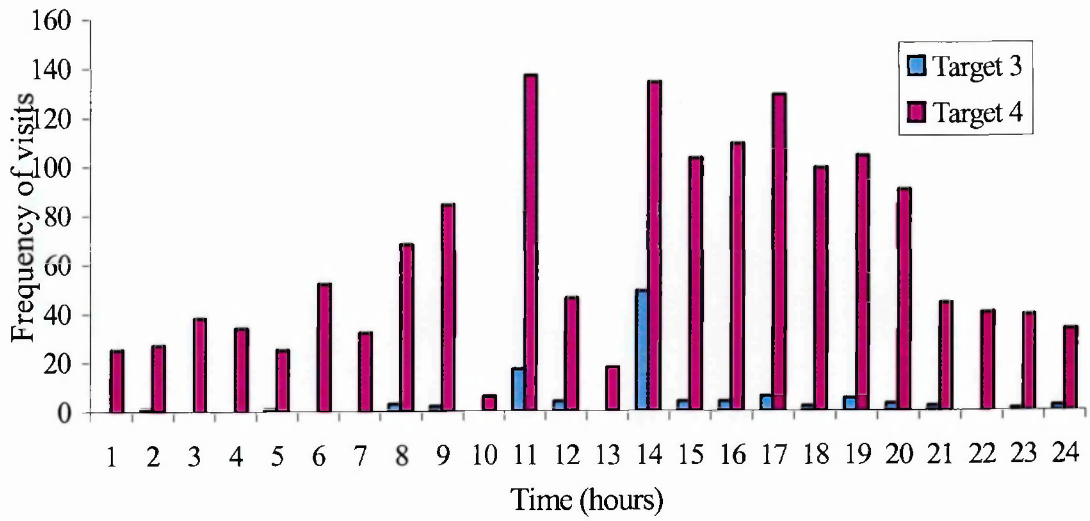

Figure 7.4 The feeding patterns of piglets mixed at 14 days of age and no access to creep pre-weaning (M14NC) when offered a choice of two diets on day 46.

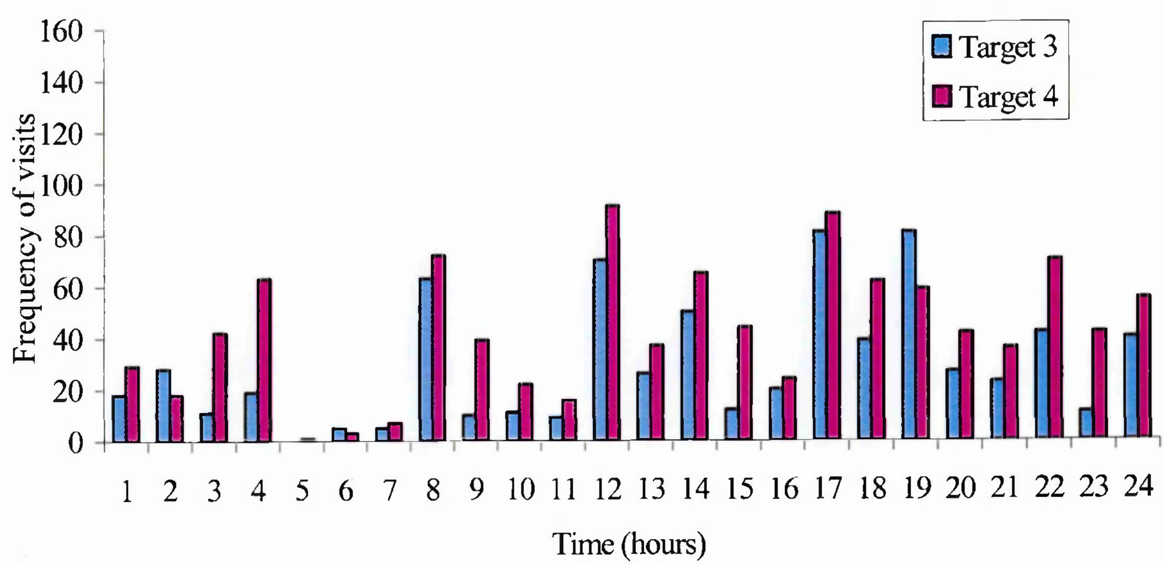

Figure 7.5 The feeding patterns of piglets mixed at 14 days of age and offered creep pre-weaning (M14C) when offered a choice of two diets on day 46.

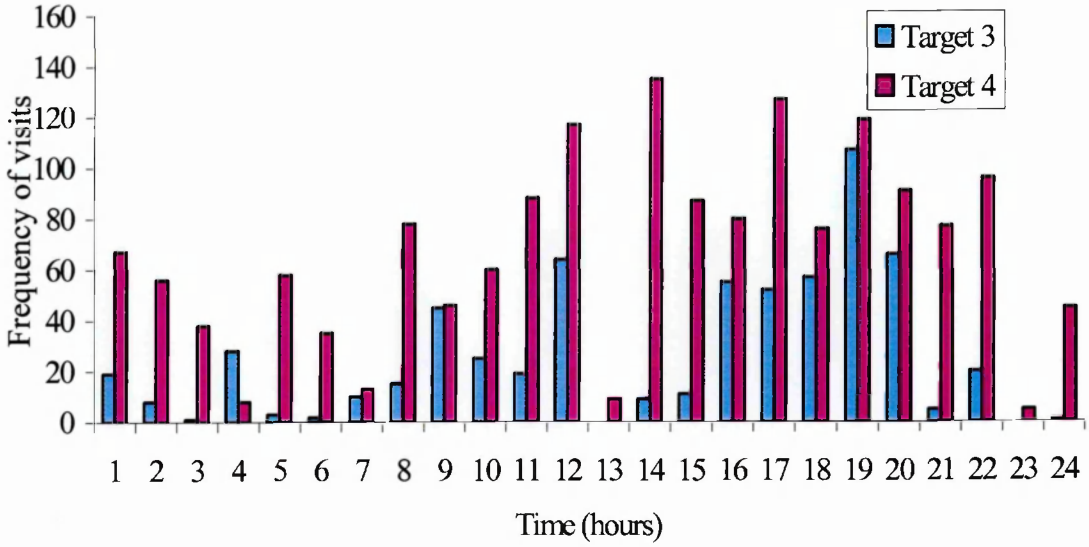

Figure 7.6 The feeding patterns of piglets mixed at weaning and with no access to creep pre-weaning (M28NC) when offered a choice of two diets on day 46.

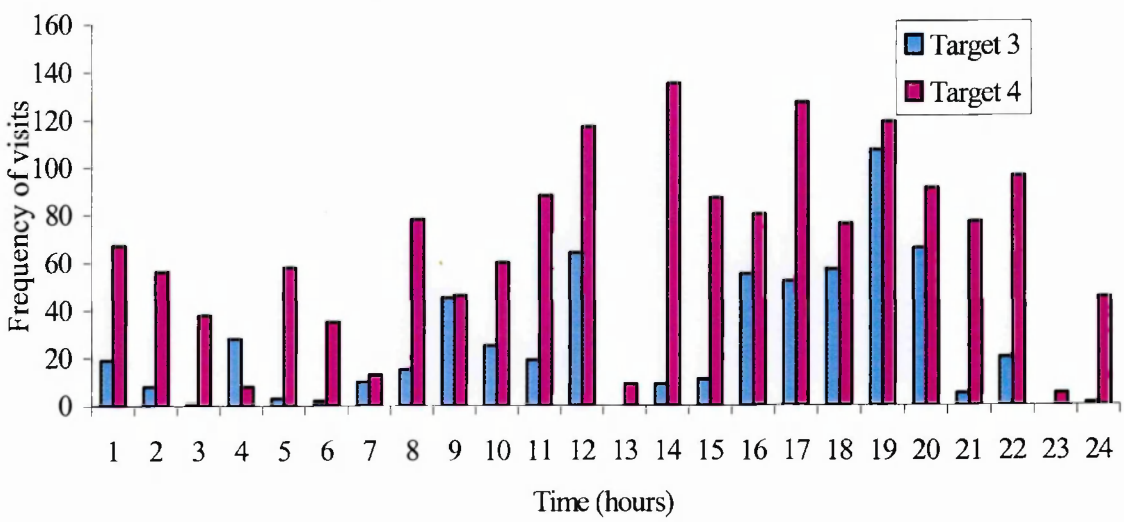

Figure 7.7 The feeding patterns of piglets mixed at weaning and offered creep pre-weaning (M28C) when offered a choice of two diets on day 46.

7.3.4 Immune responses

7.3.4.1 Humoral immune response

There was no significant mixing x creep interaction at any stage post-weaning on anti-KLH IgG_1 response (Table 7.8 and Figure 7.8). There was also no significant effect of mixing on anti-KLH IgG_1 response. Piglets offered creep had a significantly lower anti-KLH IgG_1 levels compared to piglets without creep access on day 35 (0.103 vs. 0.056 for C and NC respectively, $P<0.001$). This difference remained significant on days 42 (0.853 VS. 0.989 for C and NC respectively, $P<0.001$) and 49 (0.799 vs. 0.956 for C and NC respectively, $P<0.001$).

Table 7.8 *Effect of mixing prior to weaning and access to creep on piglet IgG_1 antibody response post-weaning (-log transformed data)*

	Treatment					Significance		
	M28 C	M28 NC	M14 C	M14 NC	s.e.d	Mix	Creep	mix x creep
IgG_1 Response (untransformed means (OD_{405nm}))								
Day 28	1.931 (0.012)	1.702 (0.073)	1.534 (0.059)	2.040 (0.005)	0.1929	-	-	-
Day 35†	1.153 (0.115)	1.460 (0.065)	1.230 (0.090)	1.623 (0.046)	0.1431	NS	<0.001	NS
Day 42†	0.096 (0.876)	0.011 (0.999)	0.102 (0.830)	0.000 (0.979)	0.0359	NS	<0.001	NS
Day 49†	0.122 (0.812)	0.023 (0.962)	0.133 (0.786)	0.016 (0.951)	0.0401	NS	<0.001	NS

† Means adjusted for day 28 as a covariate

There was no significant mixing x creep interaction at any time on anti-KLH IgG_2 during the post-weaning period (Table 7.9 and Figure 7.9) although a tendency was observed on day 35 where piglets mixed pre-weaning and given access to creep had the highest anti-KLH IgG_2 response compared with piglets mixed pre-weaning and no access to creep who had the lowest anti-KLH IgG_2 levels (P=0.052). As with the anti-KLH IgG_1 results there was a significant effect of creep feeding on anti-KLH IgG_2

levels on days 35 (0.127 vs. 0.091 for C and NC respectively, $P<0.001$), 42 (0.362 vs. 0.466 for C and NC respectively, $P=0.001$) and 49 (0.299 vs. 0.351 for C and NC respectively, $P=0.015$) where piglets with no access to creep prior to weaning had a higher anti-KLH IgG_2 responses compared with piglets with access to creep pre-weaning.

Table 7.9 *Effect of mixing prior to weaning and access to creep on piglet IgG_2 antibody response post-weaning (-log transformed data)*

	Treatment					Significance		
	M28 C	M28 NC	M14 C	M14 NC	s.e.d	Mix	Creep	Mix x creep
IgG_2 Response (untransformed means (OD_{405nm}))								
Day 28	1.170 (0.113)	1.086 (0.144)	1.033 (0.113)	1.471 (0.051)	0.1392	-	-	-
Day 35†	1.063 (0.107)	1.158 (0.098)	0.999 (0.147)	1.331 (0.084)	0.0838	NS	<0.001	NS
Day 42†	0.526 (0.335)	0.351 (0.477)	0.481 (0.389)	0.370 (0.454)	0.0604	NS	0.001	NS
Day 49†	0.554 (0.299)	0.467 (0.368)	0.596 (0.299)	0.484 (0.335)	0.0560	NS	0.015	NS

† Means adjusted for day 28 as a covariate

A significant mixing x creep interaction on anti-KLH IgM response was observed on day 35 post-weaning ($P=0.024$) where piglets mixed pre-weaning with access to creep had the highest levels of anti-KLH IgM levels compared with piglets mixed pr-weaning and no access to creep feed (Table 7.10 and Figure 7.10). There was a significant effect of access to creep on Anti-KLH IgM response on days 35 (0.339 vs. 0.276 for C and NC respectively, $P=0.008$) and 42 (0.364 vs. 0.302 for C and NC respectively, $P=0.002$) where piglets given creep feed pre-weaning had lower levels of Anti-KLH IgM compared with piglets without creep feed pre-weaning. However, this significant difference was no longer apparent by day 49.

Table 7.10 *Effect of mixing prior to weaning and access to creep on piglet IgM antibody response post-weaning (-log transformed data)*

	Treatment					Significance		
	M28 C	M28 NC	M14 C	M14 NC	s.e.d	Mix	Creep	mix x creep
IgM Response (untransformed means (OD_{405nm}))								
Day 28	1.159 (0.091)	1.265 (0.091)	1.062 (0.107)	1.267 (0.067)	0.0960	-	-	-
Day 35†	0.550 (0.310)	0.573 (0.289)	0.455 (0.369)	0.675 (0.263)	0.0612	NS	0.008	0.024
Day 42†	0.464 (0.367)	0.563 (0.303)	0.463 (0.361)	0.592 (0.301)	0.0495	NS	0.002	NS
Day 49†	0.505 (0.321)	0.524 (0.307)	0.525 (0.307)	0.507 (0.315)	0.0277	NS	NS	NS

† Means adjusted for day 28 as a covariate

A significant mixing x creep interaction on anti-KLH IgA antibody response was observed on days 42 (P=0.012) and 49 (P=0.024) yet there does not appear to be any pattern of the treatments (Table 7.11 and Figure 7.11). There was no significant effect of mixing on post-weaning anti-KLH IgA antibody response but on day 35 piglets given creep feed had significantly higher levels of anti-KLH IgA antibodies circulating in the blood compared to piglets without creep feed (0.119 vs. 0.080 for C and NC respectively, P<0.001). However, this difference was no longer apparent on days 42 & 49.

Table 7.11 *Effect of mixing prior to weaning and access to creep on piglet IgA antibody response post-weaning (-log transformed data)*

	Treatment					Significance		
	M28 C	M28 NC	M14 C	M14 NC	s.e.d	mix	Creep	Mix x creep
IgA Response (untransformed means (OD_{405nm}))								
Day 28	1.731 (0.027)	1.733 (0.048)	1.282 (0.074)	1.724 (0.019)	0.1324	-	-	-
Day 35†	1.049 (0.128)	1.189 (0.092)	0.993 (0.111)	1.395 (0.068)	0.1079	NS	<0.001	NS
Day 42†	0.421 (0.375)	0.502 (0.349)	0.533 (0.305)	0.473 (0.340)	0.0390	NS	NS	0.012
Day 49†	0.553 (0.284)	0.602 (0.281)	0.612 (0.272)	0.510 (0.313)	0.0462	NS	NS	0.024

† Means adjusted for day 28 as a covariate

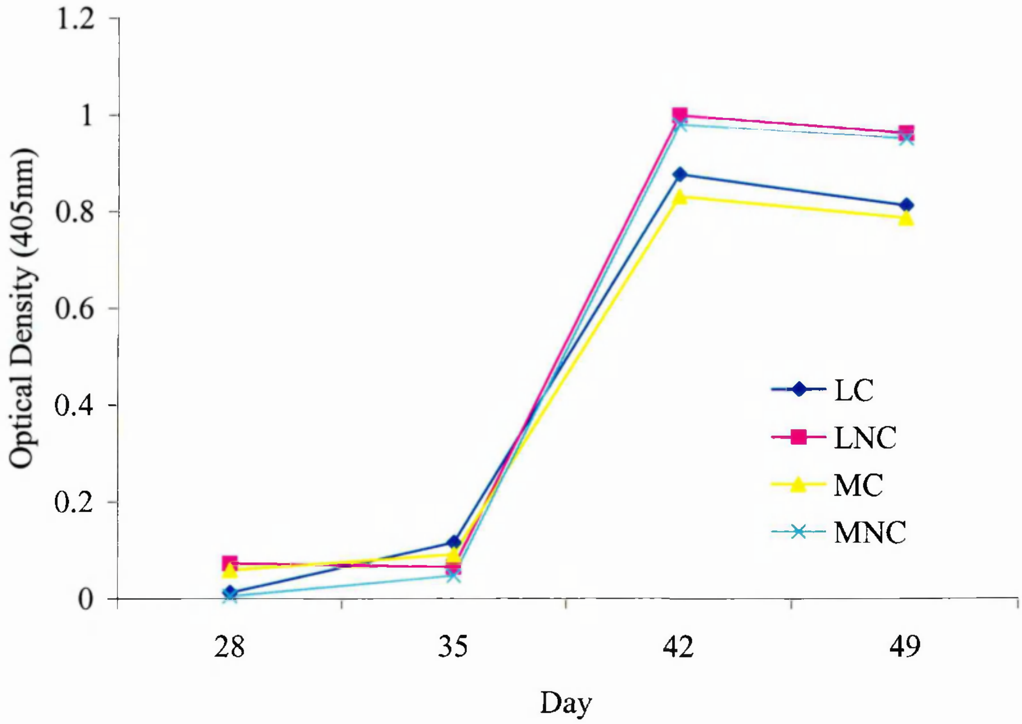

Figure 7.8 Effect of mixing pre-weaning and creep feed availability on Anti-KLH IgG_1 antibody response (untransformed means)

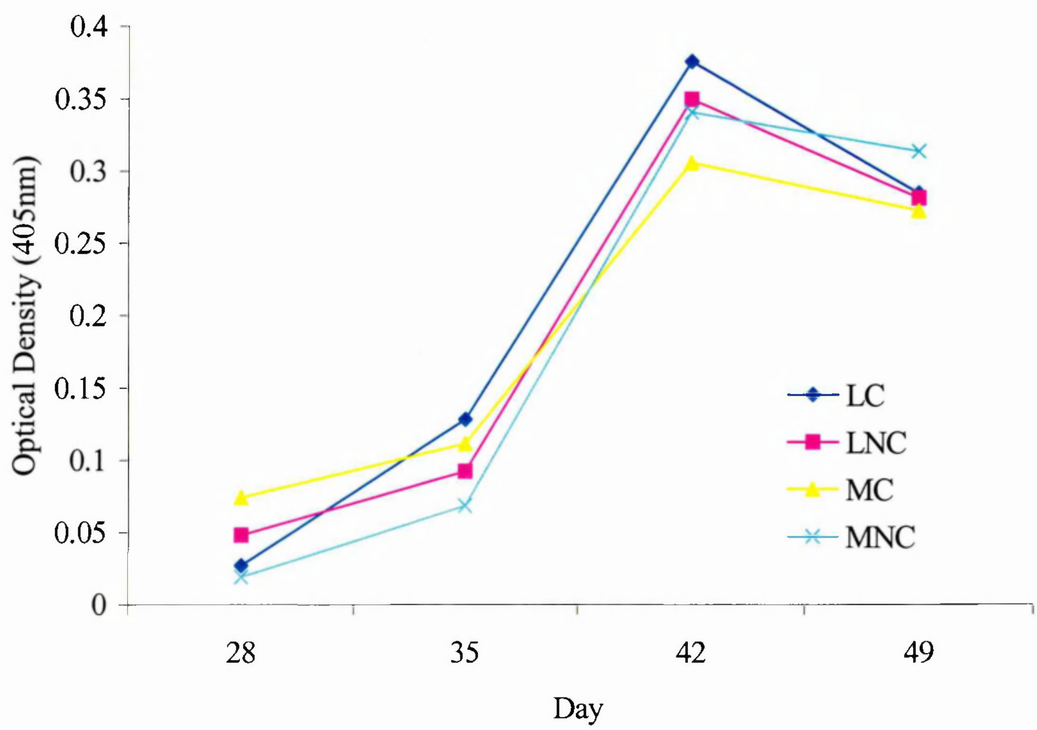

Figure 7.9 Effect of mixing pre-weaning and creep feed availability on Anti-KLH IgG_2 antibody response (untransformed means)

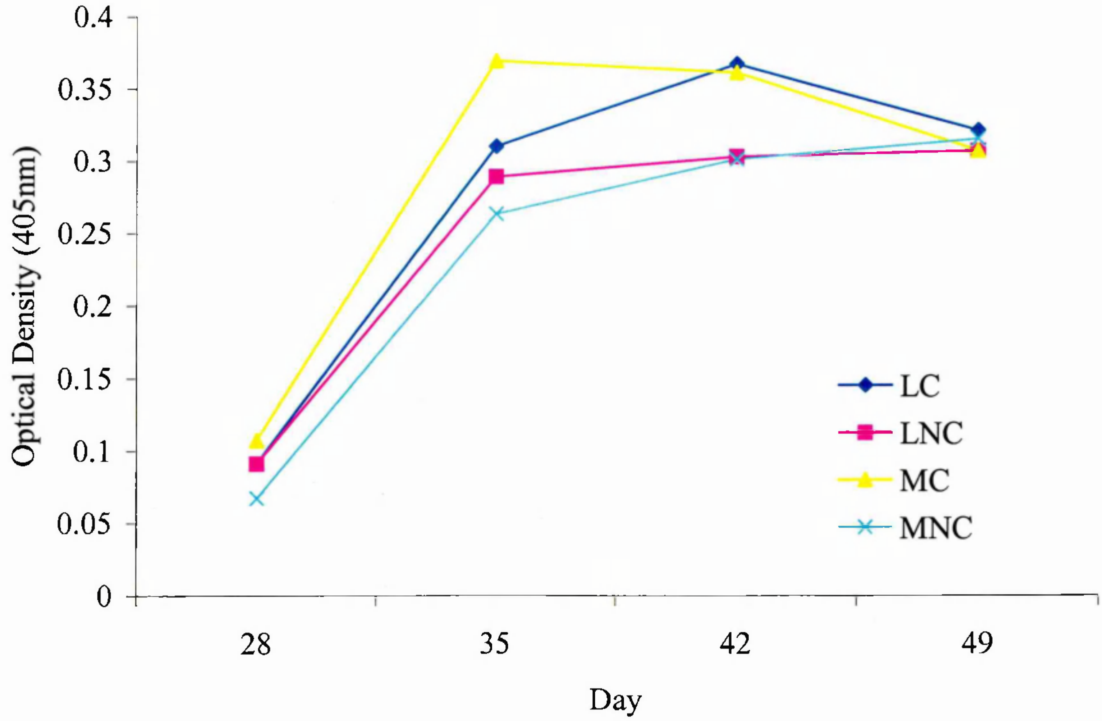

Figure 7.10 Effect of mixing pre-weaning and creep feed availability on Anti-KLH IgM antibody response (untransformed means)

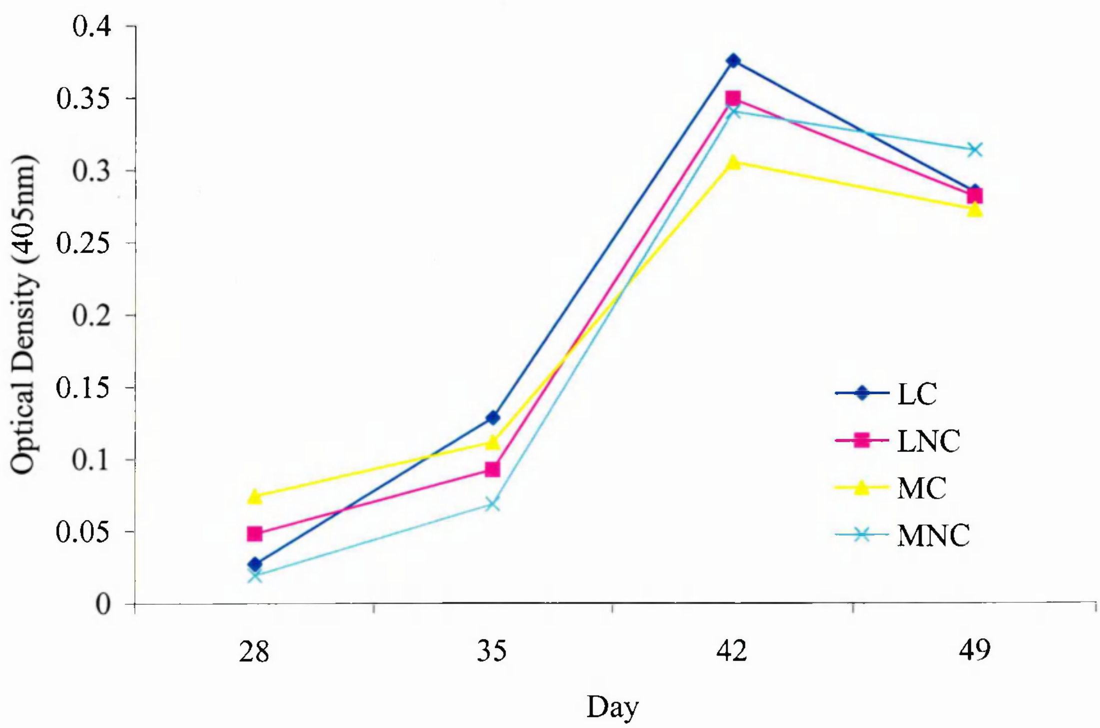

Figure 7.11 Effect of mixing pre-weaning and creep feed availability on Anti-KLH IgA antibody response (untransformed means)

7.3.4.2 Cell-mediated immunity

There was no significant mixing x creep interaction on any of the days for any of the mitogens or the control on the *in vitro* lymphocyte blastogenesis assays (Table 7.12). Piglets mixed pre-weaning had higher lymphocyte blastogenic responses compared with piglets mixed at weaning on day 49 (unstimulated - 0.068 vs. 0.095 for M28 and M14 respectively, P=0.018: Con A - 0.069 vs. 0.103 for M28 and M14 respectively, P=0.014; KLH - 0.069 vs. 0.099 for M28 and M14 respectively, P=0.046). The response to PWM was not significant but there was a tendency following the same pattern as the other mitogens (P=0.053).

Table 7.12 *Effect of mixing prior to weaning and access to creep on in vitro lymphocyte blastogenesis test responses to Concanavalin A (Con A), Pokeweed mitogen (PWM), KLH and a control (RPMI 1640) post-weaning (-log transformed data) (Optical density 507nm with reference optical density 630nm)*

	Treatment					Significance		
	M28 C	M28 NC	M14 C	M14 NC	s.e.d	mix	Creep	mix x creep
Lymphocyte response unstimulated (untransformed data OD_{507}/OD_{630})								
Day 35	1.331 (0.049)	1.187 (0.069)	1.193 (0.076)	1.202 (0.077)	0.0897	NS	NS	NS
Day 49	1.220 (0.067)	1.200 (0.069)	1.063 (0.093)	1.071 (0.097)	0.0822	0.018	NS	NS
Lymphocyte response to Con A (untransformed data OD_{507}/OD_{630})								
Day 35	1.278 (0.056)	1.163 (0.077)	1.182 (0.079)	1.153 (0.088)	0.0960	NS	NS	NS
Day 49	1.183 (0.069)	1.245 (0.069)	1.022 (0.104)	1.062 (0.102)	0.0943	0.014	NS	NS
Lymphocyte response to PWM (untransformed data OD_{507}/OD_{630})								
Day 35	1.366 (0.051)	1.262 (0.067)	1.302 (0.0630	1.307 (0.064)	0.1026	NS	NS	NS
Day 49	1.328 (0.056)	1.359 (0.052)	1.159 (0.085)	1.206 (0.084)	0.1147	NS	NS	NS
Lymphocyte response to KLH (untransformed data OD_{507}/OD_{630})								
Day 35	1.337 (0.048)	1.189 (0.071)	1.232 (0.068)	1.191 (0.080)	0.0871	NS	NS	NS
Day 49	1.196 (0.071)	1.215 (0.066)	1.051 (0.101)	1.087 (0.096)	0.0943	0.046	NS	NS

There was no significant effect of treatment on stimulation indices for any of the mitogens. The stimulation indices were calculated by the following equation used by Mosmann (1983):

$$\frac{\text{Stimulated } OD_{507}(\text{ref } OD_{630})}{\text{Unstimulated control } OD_{507}(\text{ref } OD_{630})}$$

7.3.4.3 IFN–γ assay

There was no significant mixing x creep interaction or significant main effect of mixing or creep on gamma-interferon production by isolated peripheral blood lymphocytes (Table 7.13).

Table 7.13 *Effect of mixing pre-weaning and access to creep feed on lymphocyte interferon-gamma production on day 35*

	Treatment					Significance		
	M28 C	M28 NC	M14 C	M14 NC	s.e.d	Mix	creep	Mix x creep
IFN–γ concentrations in supernatant (pg/ml)								
	82.6	45.9	47.0	52.7	20.18	NS	NS	NS

7.3.5 Gut morphology and adrenal glands

There was no significant effect of treatment on the length or weight of the small intestines or on the weight of the adrenal glands on day 33 (Table 7.14).

Table 7.14 *Effect of mixing pre-weaning and access to creep feed on piglet intestine weight and length and adrenal gland weight*

	Treatment					Significance		
	M28 C	M28 NC	M14 C	M14 NC	s.e.d	Mix	creep	Mix x creep
Intestine measurements								
Length(cm)	913	922	983	903	49.1	NS	NS	NS
Weight(g)	341	339	379	340	26.4	NS	NS	NS
Adrenal glands (proportion of final body weight)								
Left	0.060	0.060	0.063	0.065	0.0049	NS	NS	NS
Right	0.002	0.002	0.002	0.002	0.0001	NS	NS	NS

Limited numbers of entire villi and crypts were visible for accurate measurement because of poor preparation of samples which reduced the number for analysis and therefore the results have been interpreted with caution. At 25 % along the small intestine there was no significant effect mixing or creep feed on villus height or on the villus height to crypt depth ratio. However, piglets allowed access to creep had deeper crypts compared to piglets without access to creep prior to weaning at 25% (253μm vs. 211 for C and NC respectively, s.e.d 10.5, *P*=0.007).

A significant mixing x creep interaction on villus height was observed at 50% (*P*=0.015) where piglets mixed at weaning and no prior access to creep feed had significantly shorter villi compared with the other three treatments (*P*=0.015), yet there was no mixing x creep interaction on crypt depth at 50% along the small intestine (Table 7.15). Piglets mixed at weaning had shorter villi compared with piglets mixed at 14 days of age (Table 7.16) at 50% along the small intestine (306μm vs. 338 for M28 and M14 respectively, s.e.d 1.3, *P*=0.027). There was also a significant effect of creep feed on villus height 50% down the small intestine where piglets offered creep from 7 days of age had longer villi than piglets without access to creep pre-weaning (331μm vs. 313 for C and NC respectively, s.e.d 1.3, *P*=0.046). As at 25% along the small intestine there was no significant effect of mixing or creep feed availability on the villus height to crypt depth ratio (Table 7.15).

At 75% along the small intestine piglets mixed at weaning and offered creep prior to weaning had significantly taller villi compared with the other treatment (*P*=0.004) but there was no significant mixing x creep interaction on crypt depth (Table 7.15). At 75% along the small intestine piglets mixed at 14 days of age and offered creep had significantly smaller villus height to crypt depth ratio (P=0.036) compared to the other 3 treatments. At 75% along the small intestine, piglets mixed at 14 days of age had shorter villi than piglets mixed at weaning (273µm vs. 221 for M28 and M14 respectively, s.e.d 17.9, P=0.020) and piglets allowed access to creep had deeper crypts compared to piglets without access to creep prior to weaning (240µm vs. 187 for C and NC respectively, s.e.d 17.5, P=0.016).

Table 7.15 *Effect of mixing pre-weaning and access to creep feed on piglet villus height, crypt depth and V:C ratio*

	Treatment					Significance		
	M28 C	M28 NC	M14 C	M14 NC	s.e.d	Mix	Creep	Mix x creep
Villus height (µm)								
25%	373	322	324	273	46.9	NS	NS	-
50%	344	268	319	357	1.9	0.027	0.046	0.015
75%	326	220	202	240	25.3	0.020	NS	0.004
Crypt depth (µm)								
25%	252	210	253	212	14.9	NS	0.007	-
50%	268	241	227	282	15.2	NS	NS	NS
75%	237	196	244	178	24.8	NS	0.016	NS
Villus height:crypt depth ratio								
25%	1.5	1.5	1.3	1.3	0.17	NS	NS	-
50%	1.3	1.1	1.4	1.3	0.06	NS	NS	NS
75%	1.4	1.2	0.8	1.3	0.21	NS	NS	0.036

7.4 DISCUSSION

7.4.1 Performance

There were very few differences observed between the treatments throughout this trial in terms of performance data such as growth rates, feed intakes and feed conversion ratios relating to mixing or access to creep feed. These results were unexpected in light of the results previously reported (sections 3.3.1 & 4.3.1) where mixing at 14 days of age showed positive improvements in post-weaning performance but this is in agreement with Pluske and Williams (1996b) who observed no differences in growth rates or feed intakes when three litters were allowed to mix from 10 days of age and weaned at 28 days.

However, there was a difference in the genetics of the dams used in this study compared to those reported in sections 3.3.1 & 4.3.1. As a result of the constraints of restocking during the Foot and Mouth outbreak a number of homebred gilts were utilised during this study instead of the PIC Camborough 15 gilts and sows used in the previous studies. The treatments were blocked to avoid bias in the results. Another potential difference was in the shape of the weaner accommodation compared to the previous studies but the space allowance per pig was very similar (section 2.1).

Creep feed intakes have been under review for several years (section 1.6.1) with continuing dispute about the pros and cons of feeding creep pre-weaning due to the variability in intake. Feed intakes observed from day 6 to 21 were very low (13-79g/day) and similar to those of Fraser *et al.* (1994) who found low feed intakes (11-44g/day) from 14 to 28 days of age. Creep feed in other work had been offered from approximately 14 days of age and provided until weaning (Barnett *et al.*, 1989; Appleby *et al.*, 1991; Pajor *et al.* 1991).

Maintaining the piglet's interest in the creep feed seemed to be vital in encouraging piglets to visit the trough and stimulate exploratory behaviour hence by removing the feed and/or trough or replacing the feed regularly piglets may increase pre-weaning feed intakes and therefore help in improve post-weaning feed intakes (Delumeau and Meunier-Salaun, 1995).

7.4.2 Lesion scores

Lesion scores followed a very similar pattern as reported in all the previous chapters therefore supporting the conclusion that mixing piglets at 14 days of age reduces lesion scores post-weaning which indicates a reduction in post-weaning aggression. This may indicate that aggressive behaviour is taking place earlier. However, it has been shown (sections 3.3.2, 4.3.2, 6.3.2 & 7.3.2) that piglets mixed pre-weaning have lower lesion scores than those mixed at weaning. This is in agreement with Pitts *et al.* (2000) who suggests that younger pigs cannot inflict as much skin damage on their pen mates if mixed earlier in life.

7.4.3 Behavioural observations

Duration of visits to the trough was reported to increase due to interactions between piglets recently mixed familiarising themselves with the environment (Rushen, 1988) and determining the social hierarchy (Fraser, 1974; Craig, 1986). This is also supported by the increase in lesion scores post-mixing, which therefore reduced the time spent exploring the environment and feeding. Morrow and Walker (1994) identified that feeding behaviour was affected by the number and position of feeders and that the number of visits increased with increasing numbers of feeders and concluded that, ideally, they should not be positioned side by side. This was using non-pelleted diets and did not affect the duration of feeding visits although it was reported that there were two peaks of feeding activity (Morrow and Walker, 1994) whereas only one main peak was observed in this study (Figures 7.4-7.7).

There was considerable variation in feed intake during the choice feeding although there was no significant difference between the treatments in the amount of each diet selected. All treatment groups consumed more of one diet thus indicating a definite preference. This may relate to the composition of each diet available and the variation of piglet weight within each group. Previous studies (Kyriazakis *et al.*, 1990; Dalby *et al.*, 1995) also observed a short period of time when the piglets tested both diets before selecting the most appropriate diet for their requirement.

There was a difference in the presentation of the final weaner diet (Target 4) and the grower diet offered in the final week of the trial particularly in terms of pellet size of the Grower diet in comparison to the Target weaner diets. Although all of the piglets were of a sufficient weight to move onto the grower diet, there was a clear preference for the small pellet. The specifications of the diets were different yet the proximate analysis of these two diets gave very similar levels of DE, dry matter and oil content, thus suggesting that the presentation is more likely to have affected the piglet's choice rather that nutrient composition. However, the NDF was higher in the grower diet and therefore may suggest that palatability is lower as was lysine levels which alters the lysine to DE ratio.

7.4.4 Immune responses

The absence of any effects of mixing on anti-KLH antibody response due to mixing is contradictory to previous chapters (chapters 3 and 4) where clear differences between piglets mixed pre-weaning and at weaning were reported. The absence of any difference in performance and immune response confirms that for some reason, the effects of mixing pre-weaning were absent in this study. Creep feeding has not previously been assessed in relation to humoral immune response yet there was a clear effect on immunity post-weaning. Previous studies assessing changes in diets have shown enhanced antibody production to sheep erythrocytes (Dee, 1999), however as KLH induces a different type of antigen presenting cell and T helper subtype, it cannot be directly related to these results.

The effect of mixing prior to weaning on cell mediated immune response gave some significant results compared with the previous study possibly due to the different methodology used, which supports work by Blecha *et al.* (1983) and Mackenzie (1994) where stress causes a reduction in cell-mediated immunity.

Deguchi and Akuzawa (1998) assessed blastogenic responses to PWM, Con A and PHA after grouping piglets together after 64 days of age and determined that there was no effect of regrouping on lymphocyte proliferation 7 and 14 days post-mixing. This gives similar results to this study, as no effect was observed 7 days after weaning yet by 21 days there were differences between those piglets previously mixed prior to weaning. There might have been a difference in Deguchi and Akuzawa's (1998) study had they continued assessing responses past 14 days. However the age difference may also have had an effect on lymphocyte proliferation as seen by Blecha *et al.* (1983).

The ability of T-lymphocytes to proliferate is known to be dependent on their ability to produce interleukin-2 (IL-2), a known growth factor for T-lymphocytes (Bailey *et al.*, 1992). However, IL-2 production tends to be reduced at weaning and it has been reported that isolated lymphocytes do not produce significant levels of IL-2 (Cantrell and Smith, 1983), which might have had an impact on the results from this technique. Bailey *et al.* (1992) identified that using lymphocytes from alternative sites (solid organs) such as the spleen or mesenteric lymph nodes, gives improved results when assessing IL-2 production compared to isolated cells from peripheral blood. This may mean that using alternative sources of T-lymphocytes would have given different results and needs to be considered in future cell-mediated immune response assays.

The reduction in IL-2 and, potentially IFN-γ, production associated with weaning and stress appears to evoke a change in T-helper cell response with a shift from Th1 to Th2 (Rook *et al.*, 1994), thus supporting the converse relationship between cell-mediated and humoral immune responses to the same stressor (Hessing *et al.*, 1995).

7.4.5 Gut morphology

The structure of the small intestine changes dramatically post-weaning with significant increases in crypt depth and decreased villus height (Kenworthy, 1976; Hampson, 1986a; Nabuurs *et al.*, 1993) as highlighted in section 1.6 and 6.4.5. Nabuurs *et al.* (1993) identified that giving supplementary feed during the suckling period helped prevent the decrease in villus height thus reducing the risk of post-weaning diarrhoea.

The cause of the change in the digestive tract has been reported to be due to inflammatory reaction of the digestive tract to products of digestion (Kenworthy, 1976) and the lack of feed consumed post-weaning delaying the normal functions and maturation of the digestive tract plus causing an imbalance in the microflora causing increase in bacterial levels (Cera *et al.*, 1988). Another area of change appears to be sudden transition in cell production and fewer mature enterocytes being apparent thus reducing the absorptive capacity in the small intestine (Hampson, 1986a).

It is clear that there are complex changes occurring within the digestive tract around the point of weaning involving an immune response to dietary antigens and bacterial infection (Stokes *et al.*, 1994; Bailey *et al.*, 1994), alteration in glcosylation which affects interaction with microbes (Kelly and King, 2001) and general development of the digestive tract (Pluske *et al.*, 1991; Aumaitre, 2000). Therefore no single management strategy will provide a solution to the fact the digestive tract is significantly underdeveloped when weaning at 3-4 weeks of age.

7.4.6 Adrenal glands

Adrenal gland weights have been recorded previously to identify any changes in long-term cortisol levels as an indicator to stress (Hessing *et al.*, 1994). Beattie *et al.* (2000) reported that a barren environment caused increased levels of plasma cortisol and enlarged adrenal glands at 21 weeks of age compared to an enriched environment. These results were not reflected here, however, in Beattie *et al.* (2000) piglets were allocated to one of these treatments throughout the 21 weeks, and so the stressor was applied for a longer period in comparison to this trial where the stressors were only applied for a shorter period (either 7 or 21 days). There has been little work carried out on the effectiveness of this measure of stress although the results appear to support that mixing prior to weaning at 14 days of age causes no longer term stress effect on piglets post-weaning.

7.5 CONCLUSIONS

The provision of creep from an early age may help increase post-weaning intakes by allowing the piglet access to the diet for a longer period of time prior to weaning. It is clear that maintaining the interest of piglets in the feed is essential and simple techniques, such as removing the bowl for short periods of time, initiated exploratory behaviour each time the feed was returned to the farrowing pen (Delumeau and Meunier-Salaun, 1995). There are still numerous questions relating to creep feeding that need to be addressed including how it affects post-weaning performance.

The lack of improvement in performance was disappointing and does not support the previous studies in terms of mixing pre-weaning improving post-weaning performance potentially caused by the different genetics of the sows and alternative shaped weaner accommodation and/or factors that have not been identified. A continued theme throughout has been the reduction in aggression post-weaning, observed through the measurement of lesion scores, when mixing occurred at 14 days of age.

Creep feeding and mixing had significant effects on intestinal morphology post-weaning. However, it was not possible to determine if these were biologically significant as based on weight gain and FCR, they had little effect. Overall this study suggests that creep feeding from 6 days of age can be beneficial to the piglet by allowing increased feed intake and the recognition of the same diet post-weaning to aid the transition to a fully solid diet.

CHAPTER 8. THE EFFECT OF DIFFERENT WEANING MANAGEMENT STRATEGIES ON PIGLET PERFORMANCE AND IMMUNE FUNCTION – GENERAL DISCUSSION

8.1 INTRODUCTION

The aim of this research was to assess the effects of different weaning management practices on the performance, behaviour and health of piglets. The studies aimed to replicate commercial conditions at all times so that the results would be applicable to the pig producer. Weaning is a major area of cost for the producer as a result of the associated stress causing reduced growth rates (Okai *et al.*, 1976). The piglet's potential performance is limited and number of days to slaughter increased thus contributing to an increase in feed costs (Fraser *et al.*, 1998). Alternative weaning strategies such as family systems (Goetz and Troxler, 1995), multisuckling systems (Wattanakul *et al.*, 1997a) and freedom crates (Arey, 1993) have been investigated along with more drastic approaches such as specific-stress-free housing (Ekkel *et al.*, 1995) and segregated early weaning (Harris, 1990).

These studies investigated the key factors that affect the performance of the newly weaned piglet in a commercial environment and suggest relatively simple alternative management strategies. A review of the literature identified three main areas of stress associated with weaning. These are the social relationships between piglets resulting from weaning and mixing unfamiliar piglets (Petherick and Blackshaw, 1987; Algers *et al.*, 1990; Pluske and Williams, 1996b), relocation to a new environment (Bøe, 1993; Puppe *et al.*, 1997) and the dietary change associated with weaning (Varley, 1995). These three areas were selected for study and different management strategies relating to them assessed in a commercial environment.

8.2 MAIN FACTORS AFFECTING PERFORMANCE

8.2.1 Mixing

Many producers attempt to house piglets in the same groups from weaning to slaughter. However, the combination of mixing unfamiliar piglets along with the other stressors associated with weaning often impacts on growth (Fraser *et al.*, 1998) and can cause reductions in growth rates by 100 grams/day (Okai *et al.*, 1976; chapter 3-5). The concept of mixing pre-weaning has been around for several decades. It was previously common practice in Texas where it was believed to greatly reduce fighting when piglets were sorted and regrouped by weight at weaning as referred to by Olesen *et al.* (1996). This practice of removing the partitions between farrowing pens at 2 or 3 weeks of age was abandoned but no definitive explanation for the abandonment was stated (Olesen *et al.*, 1996).

Recently there has been renewed interest in this area of management. Peaceful integration of unfamiliar piglets has been reported when piglets are introduced to each other from 10 days of age in more extensive/semi-natural conditions (Petersen *et al.*, 1989; Dellmeier and Friend, 1991). As shown in chapters 3 to 6, consistent improvements in post-weaning growth rates have also been observed when piglets have been allowed to mix within the farrowing pens prior to weaning (Wattanakul *et al.*, 1997b).

The age of mixing pre-weaning is key to improving post-weaning growth. Piglets in a semi-natural environment have been observed to be introduced to the wider family group from approximately 10 days of age (Fraser *et al.*, 1998) with the reassurance of the sows presence. Results from chapter 3 showed that mixing too soon after birth reduced growth rates pre-weaning which was attributed to disruption of normal suckling patterns. Also mixing too close to weaning negated any positive effect on growth rate due to the close proximity of the stressors associated with weaning and mixing. Previous reports by Pluske and Williams (1996b) and Wattanakul *et al.* (1997b) identified 11 days of age as the most appropriate age for mixing concluding it was similar to that found in semi-natural environments. In chapter 3, 14 days of age was identified as the optimal time for mixing compared to 7 and 21 days. Although neither 10 nor 11 days were investigated in these studies, it is unlikely that differences between mixing at 10 or 14 days would affect the ontogeny of the piglet.

The impact of mixing pre-weaning on performance post weaning may be affected by the number of piglets mixed. Bryant and Ewbank (1972) reported that group size and stocking density alter the components of agonistic interactions between piglets when comparing group sizes of 6, 12 and 18 piglets although they could not find any consistency in their results as to how it impacts on agonistic behaviour. However, Turner *et al.* (2001) observed that mixing piglets in large groups (20, 60 or 80) had no effect on aggressive interactions. This suggests either that the proportion of dyadic relationships established by aggression is reduced in large groups or that the level and intensity of aggression is reduced when a greater number of individuals have to establish a hierarchy (Turner *et al.*, 2001) which can impact upon performance parameters (Turner *et al.*, 2000). McConnell *et al.* (1987) investigated the impact of group size on feed intake and weight gain and found no effect of group sizes between 8 and 24 piglets on performance parameters. In this study improvements in growth rates were greater when piglets from three litters (approximately 30 piglets) were introduced (chapters 3 & 4) compared with mixing two litters (approximately 20 piglets) (chapter 5) pre-weaning and supported by Blackshaw *et al.* (1987).

Mixing piglets twice (pre-weaning and again at weaning) may also impact on piglet performance. Mixing piglets pre-weaning at 11 days of age and again at weaning has been reported by North and Stewart (2000) to improve post-weaning growth rates compared to mixing only pre-weaning or only at weaning. However, this was not seen in chapter 5 when the double mixing at 14 days of age and again at weaning resulted in significant reductions in performance compared to mixing either at 14 days or at weaning even though accommodation and experimental design were similar in both studies.

Creep feed intake appears to be improved by mixing as observed in chapter 6. This may be the result of social facilitation (Keeling and Hurnik, 1996) as the sight of other piglets feeding encourages others to explore and consume creep. Keeling and Hurnik (1996) also reported that piglets were more likely to synchronise their feeding if they were in the presence of familiar piglets rather than strangers. Therefore piglets mixed prior to weaning should start to feed sooner post-weaning and this was indicated in the feed intakes and growth rates (chapter 3).

North and Stewart (2000) observed a tendency for increased feed intakes post-weaning when mixing twice. Piglets mixed prior to weaning had a lower incidence of agonistic interactions (chapters 3-5), as their dominance hierarchy should have been previously established. As discussed by Rasmussen *et al.* (1962) and Brouns (1993), these piglets would have used their time for exploring their new environment and feeding. Piglets are less likely to show aggression and fight with familiar piglets (Stookey and Gonyou, 1998) and thus the stress associated with weaning should have been reduced in piglets mixed prior to weaning. This corresponds with the improved growth rates in chapters 3 and 4.

Preventing or reducing the growth check (approximately a 75% reduction in growth potential immediately post-weaning in this series of studies for piglets mixed at weaning) is the key to optimising piglet performance post-weaning and improving production. Although a check was still observed in piglets mixed prior to weaning the impact of weaning on growth appears to be reduced (approximately a 50% reduction in growth potential immediately post-weaning in this series of studies for piglets mixed prior to weaning). The improvements in growth rate and weaning weight suggest real benefits for the producer of mixing pre-weaning.

However, these series of experiments also indicates that there are other stressful factors associated with weaning such as relocation, environment and dietary factors which need to be assessed and where possible, reduced in order to achieve maximum performance and health of the piglet around weaning.

8.2.2 Environmental changes

Another factor identified as a potential stressor at weaning is relocation to weaner accommodation, which is frequently a barren environment (Bøe, 1993; Hessing *et al.*, 1994). Numerous studies looking at environmental enrichment at various stages throughout the production system report varying success on improvements of performance parameters. These include the use of toys such as tyres or chains

(Schaefer *et al*, 1990; Hill *et al.*, 1998) or stimuli such as bedding, logs and branches (Petersen *et al.*, 1995; Beattie *et al.*, 2000; Amory, 2001) all of which have been reported to improve welfare and production.

Toys and other stimuli have been reported to lose their novelty value over a number of weeks and this reduced their effectiveness to enrich the environment or stimulate/maintain the interest of the piglet (Schaefer *et al.*, 1990; Petersen *et al.*, 1995). This may have implications for the current UK legislation 'The Welfare of Farmed Animals (England) (Amendment) Regulations 2003' which states that piglets must be provided with some kind of enrichment (e.g. stress balls) but not that there is any reduction in effectiveness over time. If these enrichment tools lose effectiveness over time there may need to have a number of alternatives, which can be introduced periodically (Schaefer *et al.*, 1990).

Piglets moving into a new environment at weaning have to cope with the stress of unfamiliar surroundings in conjunction with the stress of losing their mother and breaking maternal bonds, meeting unfamiliar individuals and a change in diet (Varley, 1995). Leaving piglets in the farrowing room post-weaning could potentially reduce one of the stressors associated with weaning (Puppe *et al.*, 1997).

Puppe *et al.* (1997) and Bøe (1993) reported that familiarity of environment has a positive impact on performance and behaviour as piglets coped better with weaning when in familiar surroundings. This is contradicted in chapter 6 where significant reductions in post-weaning performance, when piglets remained in the farrowing room for nine days post-weaning, was observed. However, this discrepancy can be attributed to the age of the piglets and length of time remaining in the familiar environment. Both Puppe *et al.* (1997) and Bøe (1993) weaned the piglets closer to 6 weeks of age not 24 days as in chapter 6 and kept the piglets in the farrowing environment for longer periods of time up to 9 weeks of age.

Newly weaned piglets find it difficult to maintain their body temperatures with a lower critical temperature around 27°C post-weaning (English *et al.*, 1996) and therefore need to be kept in very controlled environments, initially around 29°C and reducing gradually over time (McConnell *et al.* 1987; Nicks *et al.*, 1993). The temperature in the farrowing rooms is hard to maintain at the high temperatures that a newly weaned piglet needs to stay in their thermoneutral zone (Close and Stanier, 1984) compared with the specially designed flat deck accommodation commonly used post-weaning. The position of the heat pad and additional heat lamps restricted the piglets movement within the farrowing pen (Figure 2.1) and the additional heat provided by the sow also needs to be considered in raising the ambient temperature of the room. This may have impacted on performance as although additional heat sources were provided in the sleeping area piglets remained huddled under the heat sources to maintain their body temperature (English *et al.*, 1996) rather than feeding. This results in poorer growth rates. It has also been reported that fluctuating temperatures had an adverse effect on the growth rates of early weaned piglets (Le Dividich, 1981) and, therefore, the poor control over the farrowing room environmental temperature may have had an impact on the post-weaning performance.

It was also noted from this study that piglets did not explore the environment in the farrowing rooms possibly because of familiarity or temperature. Therefore, they did not find the feed sources supplied in the farrowing pens post-weaning. Psychologically, the farrowing pen may be associated with the sow and suckling, the piglets may have been awaiting the sow to return to feed them therefore again did not seek out an alternative food source. Removal of piglets from the sow for 7 hours per day prior to weaning has been reported not to increase creep feed intake (Wattanakul *et al.*, 1997b). Therefore the loss of the sow may be more important than the change in environment in terms of motivating the piglet to search for other food alternatives. However, piglets may have become habituated and knowing that they were returning to the sow did not therefore waste energy exploring the environment or eating creep feed that was available during the sows absence.

Jensen and Recén (1989) reported that under natural conditions, contact between the sow and piglets reduces with age until the piglets can be considered fully weaned between 16 and 22 weeks of age (Jensen, 1995). Piglets gradually become less dependent on the sow and accept that she may be absent for longer periods with less stress associated with her absence (Newberry and Wood-Gush, 1988; Jensen, 1995). Older piglets are more developed both physically and socially and are not distressed at all by the absence of the sow.

Although no data were collected, it is important to note that there was a serious impact on long-term performance of the piglets that remained in the farrowing room post-weaning. These piglets were maintained in their groups until slaughter and a significant proportion of them died due to Porcine Multi-systemic Wasting Syndrome

(PMWS) potentially indicating that the stress from remaining in the farrowing room at weaning has long term implications. The period of time between weaning and relocation may not have being long enough to deviate the stress. This resulted in increased mortality. In earlier work in chapter 3 where there was a shorter gap between mixing and weaning (21-28 days) piglets showed poorer performance.

Little research has been performed on the long term effects of relocation on performance of piglets although Rundgren and Löfquist (1989) reported that mixing and fighting had long term effects on the performance of growing pigs that was not related to food consumption or sub-clinical disease. Mackenzie (1994) observed long term effects of weaning on antibody response when calves were challenged 28 days post-weaning. This indicates that the stress of weaning has significant effects post-weaning and further work needs to be carried out to assess these long term effects so that alternative weaning management strategies can maximise performance during the growing and finishing periods.

8.2.3 Diet and creep feeding

The dietary requirements for the newly weaned piglet have been well documented (Tokach *et al.*, 1994; NRC, 1998; Thacker, 1999; British Society of Animal Science, 2003) however the management of providing these diets to get the most out of them is key in the management strategies for improving post-weaning performance. The management of the feeding regimes only was assessed during these studies. All of the diets used throughout the trials were standard commercial diets that would be used in the industry and no special additives were tested.

The provision of creep feed prior to weaning has been well researched with many contradictory studies reporting positive effects (Appleby *et al.*, 1991; Pajor *et al.*, 1991; Delumeau and Meunier-Salaun, 1995) and negative effects on piglet performance (Barnett *et al.*, 1989; Waran and Broom, 1992). The age at which to introduce creep feed and the amount and timing of providing creep on a daily basis needs careful consideration to maximise the interest in the feed and consumption by the piglets prior to weaning. Frequent provision of creep by the removal of the bowls for short periods of time has been reported to keep piglets interested and keep them coming to investigate every time the bowls are reintroduced (Appleby *et al.*, 1991).

Pajor *et al.* (2002) reported that sow-controlled (multisuckling) housing and the reduced nursing associated with this type of system lead to increased creep feed consumption, thus better preparing the piglets for weaning and improving post-weaning feed intake and hence growth rates. However, during this series of studies there appeared to be little difference in feed intakes. This may have been caused by the sows not being able to get away from the piglets in the more conventional farrowing system.

Many approaches have been made in order to increase feed intake pre- and post-weaning. These include wet/gruel diets which have been reported to increase feed intake due to the similarity in form to milk compared with pelleted feed (Blanchard *et al.*, 2000; Brooks *et al.*, 2001) and the addition of enzyme complexes including xylanase, β-glucanase and α-amylase to aid digestion of diets (Beal *et al.*, 1998; Wiseman and Simmins, 2001). Numerous strategies have been reviewed by Li *et al.* (2003) including the use of additives, acids, probiotics and flavour enhancers. They report both positive and negative benefits of each before concluding that further research is essential to find the optimum diet to aid transition at weaning for the piglet. The major limiting factor on food intake is the piglet's behavioural needs and motivation to eat and this will be discussed further in section 8.3.

As shown in chapter 7, creep feeding from an early age had no impact on post-weaning performance and there were disappointing results throughout this chapter as no effect of mixing on performance was seen either. This may have been either because of the change in genotype of some of the sows which has been reported to interact differently with nutritional input, thus potentially affecting performance parameters (Cameron and MacLeod, 1997) or because of the reduced health status of the herd due to porcine circovirus type 2 (PCV2) and PMWS. Also there was a change in flat deck accommodation compared with previous trials. There were slight variations in the diets used during the studies such as different quantities of diet provided per pig to suit the availability of the diets. This was due to trying to keep each study as close to commercial conditions as possible and therefore using the feed available at the time of each trial. This is an area for important consideration as this may have impacted upon the results and further research would be required to assess the effects of these slight variations.

8.3 MAIN FACTORS AFFECTING BEHAVIOUR

8.3.1 Mixing

The establishment of the teat order is vital for piglets to reduce aggression during suckling and allow all piglets to suckle (Rosillon-Warnier and Paquay, 1984). Disruption to the teat order and the introduction of new piglets can cause an increase in mortality as piglets often refuse to suckle from an alternative teat once the teat order has been established (McBride, 1963).

The mixing of piglets pre-weaning has previously been reported to cause increased incidence of cross-suckling (Wattanakul *et al.*, 1997b). However, mixing at 14 days of age throughout this series of studies consistently showed little cross-suckling. Piglets appeared to be able to identify their own sow and return to her whenever she called to them. Sows commonly suckle in synchrony (Wattankul *et al.*, 1997b) so all piglets are fed at a very similar time thus reducing the chance of piglets to attempt to feed from all three sows.

Alternative systems, such as family systems or multi-suckling systems may cause increased incidence of cross-suckling as the sows are free to move around and piglets may find it more difficult to identify the sow in a larger environment (Weary *et al.*, 2002). Wattanakul *et al.* (1997b) reported that relocating the sows within the farrowing crates caused increased cross-suckling as piglets returned to their home pen rather than to their mother as did Weary *et al.* (2002) in a sow-controlled/multi-suckling type system. The use of sow-controlled housing allows the sows more freedom to begin to reduce the time spent with offspring as occurs naturally. This has been reported to give lower pre-weaning weight gain (Pitts *et al.*, 2002) but this appears to be compensated by the piglets' post-weaning growth rates due to piglets coping better at weaning (Pitts *et al.*, 2002).

A link between teat order and dominance hierarchy has been reported by Scheel *et al.* (1977) and it has been suggested that it may be advantageous for the piglets to be kept with familiar piglets that they can recognise (Stookey and Gonyou, 1998). The maintenance of teat orders when piglets are mixed pre-weaning indicates that social hierarchy may not be as closely linked to teat order. Meese and Ewbank (1973) proposed that the dominance hierarchy is formed to give precedence over food; however there may be different hierarchies established over teat order, space and alternative food sources when piglets are mixed prior to weaning.

The maintenance of groups post-weaning is important if the success of mixing piglets pre-weaning is to continue and reduce disruption of the social hierarchy (Meese and Ewbank, 1973). Therefore post-weaning, piglets should remain in the same groups until slaughter, which obviously has an impact on the number of piglets to be mixed together as it maybe important to maintain the current weaner/grower/finisher system of the producer. Mixing piglets from three litters as in chapters 3, 4, 6 & 7 has been shown to produce the most stable hierarchy and reduced aggression compared with combinations of piglets from two and four litters as reported by Blackshaw *et al.* (1987).

Lesion scores have been advocated as a useful measure of aggression and fighting (de Koning, 1983) and, in this series of studies, were measured pre- and post-mixing to assess the levels of aggression when mixing occurred. It is clear that fighting by younger piglets cause fewer and less severe lesions because of their size (Pitts *et al*, 2000) and by mixing prior to weaning and maintaining groups aggression should be lower throughout the piglet's life. The majority of lesions are caused by the piglets teeth and the physical size of the piglets so it would follow that the smaller piglets would be limited in the severity of lesions inflicted. Although piglets may remain together post-weaning, fighting immediately post-weaning was still reported possibly because of the change in environment creating distress and frustration, which is released in fighting bouts between piglets (Puppe *et al.*, 1997).

Aggression at weaning has been reported as one of the main factors that causes depressed growth (Petherick and Blackshaw, 1987), therefore any method of removing this fighting should show improvements in post-weaning performance and welfare. Piglets in an established dominance hierarchy have also been reported to spend more time exploring their environment and feeding (Otten *et al.*, 1997).

The reduction in lesion scores post-weaning observed in piglets mixed prior to weaning compared with piglets mixed at weaning implies improved welfare (de Koning, 1983). However, welfare is difficult to measure and quantify (Mason and Mendl, 1993; Broom, 1997) and many different measures have been suggested, including animals preferences for certain stimuli such as environmental temperature or light and motivation to perform certain behaviours (Dawkins, 1983) or performance parameters and physiological measures (Barnett and Hemsworth, 1990). None of these measures should be relied upon as they do not correlate well (Dawkins, 1983) and, therefore, lesion scores can only be used as a general indicator of welfare but can be a useful measure of aggressive behaviour and general well-being of the piglets.

8.3.2 Environmental changes

The change in environment and the psychological stress associated with the loss of the sow has been suggested to be key in the post-weaning period (Algers *et al.*, 1990). Flat deck accommodation is renowned for being a barren environment but it allows easy environmental control and clear observation of the piglets (Meunier-Salaün and Dantzer, 1990). Enrichment of the environment has been well documented (Schaefer *et al.*, 1990; Hill *et al.*, 1998; Amory, 2001) and it has been shown to reduce post-weaning aggression and frustration often characterised as stereotypic behaviours e.g. tail biting, and redirected exploratory behaviour such as belly nosing, towards other piglets (Bøe, 1993).

A familiar environment post-weaning has been reported to reduce the stress of weaning and stereotypic behaviours (Metz and Gonyou, 1990; Bøe, 1993). However, even in the barren weaner accommodation little stereotypic behaviour was observed regardless of post-weaning environment as seen in chapter 6 where no piglets were observed tail biting or belly nosing.

Piglets did not explore the environment post-weaning when remaining in the farrowing pens, either due to familiarity or fluctuating temperatures and therefore did not seek out the troughs of feed which impacted on their performance. This may indicate that by not offering the piglets a novel environment to explore then the stress of losing maternal contact is prolonged and causes more stress than that of a new environment as discussed in section 8.2.2. The novelty of the new environment may alleviate the stress of breaking the maternal bond and encourage alternative food sources to be found. This may simulate more natural conditions where the sow would leave the piglet for longer periods over time (Jensen, 1995) and as seen in sow-controlled housing by Pitts *et al.* (2002) and Weary *et al.* (2002).

It is important to consider the piglet's needs versus motivation at this point. Animals main needs are covered by the 'Five Freedoms' identified by Brambell (1965) and revised by the Farm Animal Welfare Council in 1993. These include freedom from hunger, thirst, discomfort, pain, fear/ distress and freedom to express normal behaviour. It is important to consider that some of these are ultimate needs e.g. food and water, which must be met to allow survival of the animal and behavioural needs which are not life threatening but may cause suffering (Dawkins, 1983). As previously discussed piglets did not appear to be motivated to explore the farrowing pens for alternative food sources once the sow had been removed and therefore there must have been a more urgent need driving the piglets to remain together.

Motivation has been reported to explain why animals perform certain behaviours and involve changes in internal state (Homeostatic mechanism) causing an increase in drive to perform a particular behaviour (Tinbergen, 1951; Deutsch, 1960; Hughes and Duncan, 1988). This may explain why piglets chose to stay huddled together and maintain their body temperature above the lower critical temperature rather than feed in the farrowing room environment. The need to maintain body temperature drives the piglet to remain huddled with rest of group rather than find food particularly if the piglet is expecting the sow to return.

8.3.3 Diet and creep feeding

The relationship between creep feeding and mixing pre-weaning has previously not been reported. The phenomenon of social facilitation may cause an increase in creep consumption when more litters are kept together as there is an increased chance of piglets observing another piglet eating compared to only observing piglets from one litter (Keeling and Hurnik, 1996). However, there were no improvements in feeding behaviour (duration & frequency of visits) with mixed litters compared to individual litters as observed in chapter 7 and it did not appear that the piglets all started to eat together as suggested by Keeling and Hurnik (1996). The main behavioural observation was that removing the trough periodically and returning it after a short absence maintained the piglets interest and maximised the exploratory behaviour of the piglets as shown by Delumeau and Meunier-Salaun (1995).

Growing pigs have been reported to choose the most appropriate diet in relation to protein content for their nutritional requirements when given the choice (Kyriazakis *et al.*, 1990). Therefore, choice feeding may be a useful tool in the post-weaning period when piglets are maintained in pre-weaning groups in which individual weights are varied. This allows piglets to select the best diet for their individual needs (Bradford and Gous, 1992). Although choice feeding has previously involved a training period to allow the piglets time to familiarise themselves with the different diets, in terms of position and physiological effects of the diets offered (Gous *et al.*, 1989), Dalby *et al.* (1995) reported that a training period is not essential when utilising choice feeding. Hence, this concept has the potential to aid the problem of having to always provide for the smallest piglet in large groups of piglets post-weaning.

Social facilitation was apparent post-weaning during choice feeding and piglets appeared to start feeding together and rest together as shown in the frequency of feeding bouts over the 24 hour period observed (Figures 7.4-7.7). Piglets consistently chose the appropriate diet for their weight category except when the final weaner diet was presented along with the grower diet. Piglets chose the final weaner diet even though it was not appropriate for their weight range. This may have been as a result of the general presentation of the two diets. Edge *et al.* (2001) identified that weaned piglets would accept any size of pellet and that this did not effect growth rates or choice of feed. Therefore the choice may have been related to dietary composition of the two diets, although proximate analysis (section 7.2.3) showed little differences between them, or more likely to be due to palatability of the diets as weaner diets are formulated to be highly palatable to encourage the piglet to eat post-weaning.

8.4 MAIN FACTORS AFFECTING IMMUNE RESPONSE AND HEALTH

8.4.1 Mixing

The impact of stress on the immune systems of farm animals has been well documented (Kelley, 1980; Griffin, 1989) and the relative impact of stress caused by weaning in pigs has been considered to be greater compared to other animals because of their relative immature age at weaning (Kelley, 1980). In particular, abrupt weaning of piglets at a variety of ages has frequently been shown to cause a decrease in immune responses (Blecha and Kelley, 1981; Blecha *et al.*, 1983; Blecha *et al.*, 1985). These authors clearly stated that alterations in immune response were the result of an elevation of glucocorticoid levels and their subsequent effects. Croiset *et al.* (1987) considered the effect of a stressor on the immune response was dependent upon the time of the immune challenge in relation to the stressor, which was supported by Pollock *et al.* (1992). Therefore, in this series of studies, the time of immunisation was chosen to coincide with weaning in order to maximise any potential difference in the effect of weaning strategies on immune function.

The main trend from this set of studies was that piglets conventionally weaned showed increased humoral responses compared with piglets mixed earlier in life. This supports previous studies on the effects of weaning on calf humoral response where Pollock *et al.* (1993) and Mackenzie (1994) observed increased antibody responses associated with the stress. The same antigen and adjuvant challenge was used (KLH precipitated on alum) in these studies, however Blecha and Kelley (1981) and Blecha *et al.* (1983) who found weaning decreased humoral responses used foreign erythrocytes in their studies. Alexander and Brewer (1995) considered that the processing of these antigens was different in the subset of T helper cell that initiated the B-cell responses. Erythrocytes provoke a Th1 response whereas KLH/alum provoked a Th2 response, which may explain the difference in results obtained. The balance in the T helper response has been shown to be affected by stress in favour of promoting a Th2 response and inhibiting a Th1 response (Rook *et al.*, 1994).

Cell-mediated immunity is reported to be suppressed by stress and there is a clear converse relationship between humoral and cell-mediated immune responses to a stressor (Hessing *et al.*, 1995). Potentially this relationship is brought about by shift in T-helper response from a Th1 to a Th2 response altering the arm of the immune system that responds (Rook *et al.*, 1994). The Th1 response initiates the cell-mediated immunity whereas the Th2 response is related to the humoral antibody response and this is supported by the converse relationship observed between lymphocyte blastogenic responses and antibody response measured in chapters 4 and 7.

8.4.2 Environmental changes

The stress associated with leaving piglets in a familiar environment compared to relocating them to a novel environment is supported by the humoral immune response measured post-weaning (chapter 6). The inconsistency of temperature in the farrowing rooms may have been an additional stressor along with the social frustration of the loss of the sow impacting on the piglet causing increased stress. Previous research by Crenshaw *et al.* (1986) reported no such effect of temperature or weaning on immune response. However, subclasses of immunoglobulins were not measured individually and the effect of relocation only altered the response of IgG_1 and IgG_2 in this study. This may not have been picked up by measuring total antibody response (Rook *et al.*,

1994) or the use of different antigens may cause different responses (Roitt *et al.*, 1998).

The general health of the piglets left in the farrowing room post-weaning was poor although there were significant improvements in growth rates once relocated to flat deck accommodation (chapter 6). However, long-term health was affected as many piglets suffered from PMWS in the growing period and mortality in these groups was significantly higher than those relocated at weaning as previously discussed in section 8.2.2.

8.4.3 Diet and creep feeding

Creep feeding has been reported to be beneficial to piglets but may also be detrimental, in terms of altering gut morphology by shortening villi and increasing crypt depth, therefore affecting absorptive capacity or by priming the digestive tract to cope with solid feed (Pluske *et al.*, 1991; Nabuurs *et al.*, 1993). The impact of weaning on the digestive tract is complex. It appears that the two key factors determining length of adaptation are age at weaning and post-weaning diet composition (Fan, 2003). Many other factors have been identified and discussed previously such as inflammatory responses to feeds post-weaning (Kenworthy, 1976; Bailey *et al.*, 1994), lack of feed due to piglets fasting (Cera *et al.*, 1988) or transient hypersensitivity to dietary antigens (Miller *et al.*, 1994). Evidence of transient hypersensitivity to soya antigens was identified by a specific immune response observed in chapter 4.

The provision of creep feed from an early age did not appear to alter the morphology of the digestive tract and changes in villus height and crypt depth were associated with both piglets with access to creep and those without (chapter 7) as reported by Nabuurs *et al.* (1993). The digestive tract appears not to be able to cope with the dramatic change from a liquid to a solid diet. This often leads to increases in bacterial levels in the microflora (Cera *et al.*, 1988) and this combined with mucosal immune responses (Stokes *et al.*, 1994) reduces absorptive capacity of the digestive tract (Hampson, 1986a). The use of liquid diets or gruel has been reported to increase feed intakes immediately post-weaning and preserves the intestinal structure (Li *et al.*, 2003).

The complexities of the development of the digestive tract and the associated immune responses cannot be altered significantly by one single management strategy and significant research needs to be carried out to identify any methods of alleviating the impact of weaning on digestive physiology.

8.5 THE WEANER PIG: PHYSIOLOGICAL RESPONSE TO WEANING

The stress associated with commercial weaning can never be completely removed due to the psychological stress of the breaking of maternal bonds at such an early age regardless of the other stressors linked with the point of weaning (Varley, 1995). However, there is evidence that certain stressors occurring together have an additive effect (Hyun *et al.*, 1998) and therefore to remove or separate any of these stressors may reduce the stress associated with weaning.

The responses to stress commonly observed are dependent upon the piglet's ability to cope with acute stressors (Dantzer and Mormède, 1983) and the activation of the hypothalamic-pituitary-adrenal (HPA) axis or the sympathetic-adrenal medullary (SAM) axis. Generally, in terms of weaning, the stress is chronic and appears to trigger the HPA axis (Dantzer and Mormède, 1983). The increased levels of cortisol causes changes to the body and other hormones suppressing brain mechanisms leading to numerous problems such as fasting and reduced immune function which is already limited at weaning (Breazile, 1987).

The immune system is clearly affected by stress and closely related to the central nervous system and endocrine system (Khansari *et al.*, 1990). It has been linked to behavioural responses to stress (Hessing *et al.*, 1995) and behaviour coping strategies. Passive responses have been reported to lead to enhanced humoral responses to novel antigens (Schrama *et al.*, 1997). Increases in plasma cortisol levels around the time of weaning have been reported by Blecha *et al.* (1985) and Brown-Borg *et al.* (1993) and linked to regrouping by Moore *et al.* (1994). This increase in cortisol levels coincides with reduced cell-mediated immune responses (Westly and Kelley, 1984;

Brown-Borg *et al.*, 1993; Deguchi and Akuzawa, 1998) and therefore indicates that stress plays a key part in the piglet's ability to cope effectively with the chronic stress of weaning.

Dybkjaer (1992) highlighted certain behavioural indicators that can be used to identify stress such as play behaviour or stereotypic behaviours (redirected oral behaviours). These behavioural indicators may be useful in detecting stress in newly weaned piglets and altering management strategies before there is significant impact on the health of the piglets. Stress can be assessed in numerous ways but it is important to consider that no single indicator shows the full impact of stress or even the type of stress. For example, stress has been reported to increase resistance to specific diseases (von Borell, 1995). A review of the complex relationships between the physiological response to stress shown in immunological, behavioural and endocrine responses indicates that the significance of stress on individuals in production systems needs to be assessed against physiological, health, production and potentially reproductive criteria (von Borell, 1995).

The main factors associated with weaning are related to the management of individuals. Therefore by altering management strategies, and understanding how piglets adapt to their environment, as highlighted in the previous chapters, should reduce the stress of weaning and lead to improvements in production (Stott, 1981).

8.6 IMPLICATIONS OF ALTERING MANAGEMENT STRATEGIES

The practicalities of these different management techniques are governed by a number of factors and their uptake by the commercial producer is clearly dependent upon them. The initial area for consideration by the producer is inevitably cost. All of the management strategies assessed throughout this work were based upon low cost changes for the producer. The practical application of these techniques were key in their development therefore cost of using any of these techniques is minimal.

Much of the present research into improving post-weaning performance is based around changes in environment and different accommodation such as the family system and the multi-suckling system. These systems show significant improvements in performance, as much as 100g/day from 6 weeks of age onwards (Hatet *et al.*, 1994). Both systems require large spaces for groups of sows and complete renovation of current buildings if using a conventional production system. Therefore there are numerous costs associated with changing to these systems. The introduction of mixing piglets pre-weaning requires no change of facilities only to remove boards between farrowing pens and increase size of current weaner accommodation. From Chapters 3-6, it can be seen that comparable improvements in growth rates post-weaning are also seen using this management strategy without the expense of altering the farrowing accommodation.

It is clear that leaving piglets in a familiar environment of the farrowing house had an adverse effect on piglet performance and health. Therefore this practice is not a good management strategy for producers and continuing the practice of relocating at weaning to alternative accommodation is recommended.

The need for good stockpersons is vital in getting good performance out of piglets at weaning (Hemsworth *et al.*, 1999). There is little additional work required in terms of mixing pre-weaning although the creep feeding strategy involves additional time to maintain the piglets interest in the creep feed whilst in the farrowing room. The creep feeding from an early age may have additional costs associated with it in terms of more creep feed being required, yet if the benefits are observed later in the production system in terms of feed efficiency and days to slaughter, the cost may be offset.

8.7 OVERALL CONCLUSIONS

Mixing piglets prior to weaning at 14 days of age is a positive step to aid the transition of weaning that is easy to carry out in commercial situations without high costs. It requires little adaptation of current systems and the benefits in performance and immune function are clear. However, the timing of mixing pre-weaning is significant as mixing at 7 or 21 days of age does not show the same improvements in performance that are seen by mixing at 14 days. Although mixing at 7 days further reduces aggression post-weaning without an increase in lesion scores at 7 days of age.

The number of litters mixed also appears to impact on the benefits observed in terms of performance parameters as shown by the reduction in growth rates when mixing two litters as opposed to three litters. It is also apparent that maintaining piglets in these groups post-weaning, and potentially throughout the production system, reduces the aggression observed when dominance hierarchies need to be re-established after repeated mixing.

The use of the farrowing room as a familiar environment is not beneficial and possibly detrimental to the piglet, because of the psychological stress associated with loss of the sow and difficulty in controlling the temperatures leading to lack of motivation to search for food sources. The familiar environment appears to cause additional stress at weaning and therefore continuing the practice of relocating to weaner accommodation is key to improving performance and health. The maintenance of piglets in their groups post-weaning reduces the levels of aggression and the lesions commonly observed regardless of post-weaning environment.

It is clear that there are many areas of uncertainty surrounding the benefits of creep feeding and its impact on digestive physiology. The underdeveloped digestive tract cannot be forced to develop earlier and therefore more research is required in this area. Further studies need to concentrate on the effect of creep feeding and weaning on the digestive tract to identify techniques that reduce the impact of weaning on gut morphology to truly improve post-weaning performance.

The main theme of this study is that management strategies can be employed to reduce the stress of weaning and improve post-weaning performance although it is not possible to completely remove the growth check associated with weaning. It is important that every effort is made to alleviate the stress at weaning to improve the health and welfare of the piglet and performance for the producer.

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APPENDIX 1

STANDARD OPERATING PROCEDURE FOR WEIGHING

IN THE FARROWING ROOM:

1. All weighing took place in the morning between 7-9am
2. Weigher checked with 5kg and 10kg standard weights
3. A litter of piglets weighed individually
4. Weigher checked with 5kg standard weight
5. Repeat steps 2-3 until all litters have been weighed
6. Check weigher again with 5kg and 10kg standards weights

IN WEANER ACCOMMODATION:

1. All weighing took place in the morning between 9am-12pm
2. Weigher checked with 10kg and 15kg standard weights
3. Pen of piglets weighed individually
4. Weigher checked with 5kg or 10kg standard weight
5. Repeat steps 3-4 until all piglets have been weighed
6. Check weigher again with 10kg or 15kg standard weights